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METABOLISM OF CALCIUM IN RAT SOFT TISSUES USING Ca^{45} AS A TRACER

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The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled Metabolism of Calcium in Rat Soft Tissues Using Ca^{45} as a Tracer, submitted by Ghulam Muhiuddin Abdus Sukur Khan in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

A satisfactory method for the estimation of the element calcium by spectophotometry and the estimation of radioactive calcium (Ca^{45}) in the same sample is described. The mean recovery of radioactive calcium in standard samples was found to be 87.1 ± 3.1%. This method has been published (147).

A study was made of the effects of hypophysectomy, adrenalectomy and thyroparathyroidectomy on calcium concentration and Ca^{45} incorporation in a number of tissues of the male rat. The tissues studied were the whole blood, adrenal, dorsolateral prostate, ventral prostate, liver and heart. The effects of the administration of adrenocorticotrophic hormone (ACTH), growth hormone (STH), desoxy-corticosterone acetate (DCA), parathyroid extract (PTE) and thyroid extract (TE) to the normal and experimental animals were also observed.

A postulation has been made that calcium metabolism in the adrenal, dorsolateral prostate and ventral prostate is related to hormone activity of the pituitary gland. It is also suggested that calcium metabolism is related to the release of steroid hormones from the adrenal gland.

That the metabolism of calcium in dorsolateral and ventral prostate is independent of both thyroid and parathyroid activity is suggested from the results obtained in the thyroparathyroid experiments using thyroparathyroidectomised animals.

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INTRODUCTION

Calcium is one of the most important elements in the body. Skeleton and teeth contain ninety-nine percent of total body calcium and the remaining one percent plays vital roles in cellular and physiological activities.

The role of calcium in bone structure has been investigated very thoroughly. Advances in the knowledge of the physiology and biochemistry of bone formation during the last two decades have been remarkable. The metabolic changes in calcification appear to follow the carbohydrate phosphorylytic cycle as is found for other minerals in the body (1). The fate of calcium in bone formation has also been investigated in detail from the viewpoint of both diet as well as the influence of hormones (2, 3).

The participation of calcium in a number of functions in extra-skeletal tissue has been studied. These functions include its involvement in cell permeability, muscle contraction, blood clotting and maintenance of bioelectric potentials, and participation in certain enzyme systems.

The effect of hormones on calcium concentration and metabolism in soft tissues has not been well studied. There are few references in the literature on the effects of hormones on radio-calcium (Ca^{45}) uptake and exchange in soft tissues (4, 5, 6), all of which emphasize the necessity of controlled experiments. The amount of carrier calcium administered with Ca^{45} is very critical.

Talmage, Kraintz and Kraintz (7) have reported the distribution of Ca^{45} in a few selected tissues such as blood, seminal vesicle, kidney, dorsolateral prostate and ventral prostate. They have stated that the tissue activity approximated but never exceeded the radio-activity of blood at any given time.

The present studies were undertaken to evaluate the pattern of distribution and also to determine the uptake of Ca^{45} by various tissues in normal, adrenalectomised, hypophysectomised and thyroparathyroidectomised rats with the hope that further light might be thrown onto the effects of corresponding hormones on calcium metabolism.

The tissues of particular interest in this study were whole blood, adrenal gland, dorsolateral prostate and ventral prostate.

The work presented here includes a study of methods for the simultaneous determination of the concentration of the calcium ion and the concentration of radio-active calcium. The radioisotope Ca^{45}, which has a half-life of 152 days, and emits a weak β particle with an energy of 0.56 Mev., was used in these experiments.

The animal experimentation was divided into three series. In the first series hypophysectomised animals were compared with normal animals and both normal and hypophysectomised animals which had received an intraperitoneal injection of adrenocorticotrophic hormone (ACTH) and a further group which were administered growth hormone (STH). The second series was made up of animals which had been adrenalectomised. These were compared with normal animals and with both normal and adrenalectomised animals which had received desoxy-corticosterone acetate (DCA).

The third series included animals which had been thyroparathyroidectomised. This group of animals was compared with normal animals and with animals of each group receiving either thyroid extract (TE) or parathyroid extract (PTE).

A discussion of the conclusions and hypothetical interpretation of the conclusions are also presented.

LITERATURE SURVEY

I. CALCIUM CONCENTRATION

In 1935 Bessay and Sherman (8) found that the skeletal system contained approximately 99 percent of the total amount of calcium in the body of normally developed adult rat. Spray and Widdowson (9) found that a large increase in the amount of calcium occurred during the suckling period, after which the rate of accretion gradually fell until the mature percentage was reached.

Katz (10) was the first to give accurate figures for tissue calcium of human muscle. Burns (11) gave figures for rat tissues and those of other animals. The concentration in skin has been determined by Brown (12). Hammel (13) was the first to produce accurate ash figures of bone calcium. Forbes (14) has given the most recent figures for human bones.

A great deal of work has been done on the effect of diet and other factors upon the calcium concentration of the body; most of the work being done on rats (15). Burns (11) found no change in muscle calcium after the animals were maintained on high calcium phosphorus ratio diets. Dixon (16) found that parathyroid tetany did not affect the muscle calcium. Linder (17) reported in rats that a rachitogenic diet raised the brain calcium but left the liver calcium unchanged. Danis (18) reported that giving calcium salts apparently has little effect on the calcium concentration of tissues.

The exact state of tissue calcium is not known, but it would appear that it is bound in some fairly stable way (19).

II. INTESTINAL ABSORPTION OF CALCIUM:

Until comparatively recently there was a wide difference in opinion as to whether or not calcium salts are absorbed from the intestine in sufficient amount to cause an increase above normal in the level of serum calcium. Blau, Spencer, Swernow and Laszlo (20) using the isotope dilution method of Vissek, Monroe, and Comar (21) found the absorption of ingested calcium to be between 44 and 67 percent. Whereas, Vissek et al (21) found the "true digestibility" of calcium in cattle was from 2 to 56 percent. Gessberger (22) has reported similar results in human beings using Ca^{45}.

Calcium absorption takes place chiefly high up in the small intestine. Bergein (23) and Adolf and Liang (24) found in rats by direct analysis that calcium was absorbed in the small intestine. Nicolaysen (25) and Robinson, Stewart and Lucky (26) working with loops of intestine or Thiry-Vell fistulae found that the rate of calcium absorption was increased with increased concentration of calcium in the intestine. Innes and Nicolaysen (27) found that removal of the caecum did not affect calcium absorption. Jansen (28) observed that urinary excretion and intestinal absorption bore a relationship to each other which McCance and Widdowson (29) confirmed. Nicolaysen (30) suggested that, in rats the intestinal absorption of calcium was a function of calcium saturation of the body, and this was governed by the endogenous factor, vitamin D. McCance and Lehman (31)

have reported a marked depression of calcium retention when phosphate was added to the diet. However, two recent studies have shown that the ingestion of large amounts of phosphorus as phosphate or H_3PO_3 has no effect upon the calcium balance (32). There is continuing debate on the role of phytates in calcium balance (33, 34, 35, 36). There is little evidence of any endocrine control of calcium absorption. Soffer (37) suggested that the adrenal cortical hormones may increase the faecal loss of calcium.

The active transport mechanism has been studied in vitro with everted gut sacs and with slices of intestine (38, 39, 40, 41). In common with other mechanisms the transport of calcium is dependent on oxidative phosphorylation and is limited in capacity. The intestinal transfer requires vitamin D in the diet and is maximal in the proximal duodenum in the rat and rabbit. It varies according to the requirement of the organism for calcium. Studies with rats demonstrated that growth, pregnancy, and a diet low in calcium considerably increased transfer of this cation by everted gut sacs in vitro (39, 40).

The exact way in which dietary calcium influences the intestinal absorption of the mineral is unknown. Nicolaysen and Malm (42) suggested that low-calcium diet increased absorption by stimulating the formation of an unknown "endogenous factor" (40). In as much as calcium deprivation stimulates parathyroid secretion (43), it has been suggested that the parathyroid hormone might be the principal endogenous factor (40). However, this hypothesis which was initially supported by observations that parathyroidectomy diminished the active transport of calcium by gut sacs (40, 44), appears now to be invalid (45).

III. EXCRETION OF CALCIUM:

Calcium is lost from the body by three ways; from the gut, the kidneys and the skin. The latter is the least important but is by no means an insignificant route. Mitchell and Hamilton (46) found sweat to contain 2-7 mg. per 100 ml. Johnston and Evans (47) reported that 4-14 mg. per hour could be lost from the skin in high temperatures.

The mechanism of calcium excretion by the kidney has been investigated by a number of workers (48, 49, 50). In a series of important papers, Chen (51) and Chen and Newman (52) have reported findings on the handling of calcium by renal tubule. The calcium percentage in the glomerular filtrate was almost the same as that in an ultrafiltrate of serum (the ionized calcium fraction), reabsorption was an active process and over 99 percent of that filtered was reabsorbed. The small but constant amount of calcium excreted was considered to be an unionized complex, since calcium clearance was increased by the injection of phosphate or ethylene diamine tetra-acetic acid (Versene).

The process of reabsorption is different from that which acts on sodium or potassium (52). Calcium reabsorption is depressed by phlorizin, dinitrophenol or sodium-azide, none of which affects sodium or potassium reabsorption. On the other hand, diamox (a carbonic anhydrase inhibitor) increases sodium and potassium excretion but has no effect on that of calcium. Phlorizin and dinitrophenol inhibit phosphorylation reactions and the generation of high energy bonds. Both they and sodium-azide interfere with secretory activity of the tubules; thus some kind of metabolic energy is needed for the reabsorption of calcium.

The percentage of total calcium excretion which appears in the urine has been stated by some investigators to be fairly constant under normal conditions (53, 42, 54). After hypophysectomy the urinary calcium excretion falls in humans (55), but this may not be due to loss of a direct action of the pituitary since adrenocorticotrophic hormone, cortisone, and desoxycorticosterone cause a negative calcium balance (56) and cortisone increases the urinary and faecal calcium (57). The thyroid has a marked action on calcium excretion in the urine and in hyperthyroidism there is considerable calcium loss (58). Robertson (59) has proposed that the renal threshold for calcium is lowered, and the excessive calcium loss caused by 'Vis a fronte' action a decalcification of bone. Recent studies have shown that parathyroid hormone decreases the urine concentrating ability of the kidney in rat and man, independent of calcinosis of the tubules (60).

The faecal calcium in man consists largely of unabsorbed food calcium. Geissberger (61), and Bellin and Laszlo (62) injected Ca^{45} into humans and found the isotope to be rapidly eliminated in the faeces in amounts of from 2.5 to 15 percent of the dose. Geissberger (61) stated that four days later there was more Ca^{45} in the faeces than in the urine.

Animal experimentation suggests that they too can excrete calcium in faeces. Thus rats given Ca^{45} by injection excrete a considerable proportion of the dose in the faeces (63, 64, 65). Similarly, cats and dogs have been shown to excrete calcium by this route (66, 67).

IV. THE CALCIUM OF THE BLOOD.

The calcium concentration of blood in man is fairly constant under normal physiological conditions and is virtually all present in the plasma (or serum) (68). This constancy is seen in many other animals, but in the rabbit the plasma level is subject to great variations (68). The blood-cell of ox, sheep and rabbit unlike those of man and pig, contain variable amounts of calcium (68).

The serum calcium is in three forms: ionized, protein bound and combined with citrate and other organic substances. The ionized calcium is diffusible whereas the calcium-proteinate is not diffusible and the citrate complex is not ionized but diffusible.

The ionized fraction of plasma was originally measured by perfusing a frog's heart with the fluid to be analysed and measuring the amplitude of contractions (69). The total diffusible calcium has also been measured by ultrafiltration (70, 71, 72). In normal plasma the ionized calcium is about 65 percent of the total and ranges from 5.9 to 6.5 mg. per 100 ml. of plasma (73); most of the remainder is protein-bound; the amount of diffusible unionized calcium, measured by subtraction, is very small-0.5 - 1 mg. per 100 ml. (74, 72, 70).

The diffusible unionized calcium has not been widely studied. The non-diffusible fraction is accepted as being calcium-protein complex. McLean & Hastings (74) showed beyond doubt that a relationship exists between the calcium-ions and the protein of plasma and have calculated mass-action equations and an ionization constant. The ability of blood protein to bind calcium has been demonstrated by a number of workers.

McLean and Hastings (75) first attempted a quantitative study with purified albumins prepared from sera of horses and oxen. Martin & Parkin (76, 77) and Carr (78), reported the figures for such binding powers. Armstrong et al (79) used Ca^{45}, and reported that protein binding did not impede the transcapillary movement of calcium and that the redistribution of calcium between the ionized and bound forms was rapid.

An extreme example of the demonstration between the values for the total calcium and the ionized calcium is found in birds. In laying birds and birds given estrogens, the plasma calcium rises to very high values. The increment represents calcium going from bone to the oviduct. This calcium is combined with a soluble phosphoprotein and is not ionized (80, 81).

Starvation usually has little effect on calcium level in blood, but lengthy starvation will cause the blood calcium to fall (82). Greenberg and Miller (82) found that a calcium-deficient diet fed to growing rats caused a fall in blood calcium level after 50 days. Symptoms of tetany were not observed. In animals with well-marked phosphorus-rickets, the blood calcium level is usually a high normal and the blood inorganic phosphorus greatly lowered. When such animals are starved, the blood inorganic phosphorus rises rapidly, due to mobilization of phosphorus from soft tissues and the calcium simultaneously falls at a fast rate, and the animal is liable to tetany (83). When animals on a low calcium diet are starved, the blood calcium level remains at a constant low level for as long as 10 days (84).

V. CALCIUM AND VITAMINS.

The only vitamin with profound effects upon calcium metabolism in the body is vitamin D and associated compounds. Opinion is virtually unanimous to the effect that vitamin D promotes calcium absorption. It is also commonly accepted that increased absorption and retention of phosphate, a phenomenon prominent when experimental or clinical rickets is treated with vitamin D, is secondary to the effect of the vitamin on the absorption of calcium. Greenberg (85) performed tracer experiments with Ca^{45} and Sr^{90} and observed that vitamin D promoted absorption of calcium from the digestive tract. He interpreted his data as indicating that vitamin D also exerts a direct effect on mineralization of bone in rachitic rats. Underwood et al (86) observed that the bones of rachitic rats, given vitamin D, showed an increase in the rate of Ca^{45} deposition between the 48th and 72nd hours, without an increase in serum Ca^{45} at that time. From the work by Greenberg and Underwood, cited above, there is strong evidence that vitamin D has a direct action on bone in addition to its action on intestinal absorption.

The calcemic effect of vitamin D has been used chiefly in the treatment of tetany and very large doses of the vitamin are used. Here the effects are caused by the destructive action upon bone (87). Other substances allied to vitamin D have been used, especially dihydrotachysterol (A.T. 10). McChesney and Messer (88) found, using massive doses in dogs, that D_3 caused a longer lasting hypercalcemia than D_2, taking up to 26 days to subside, and A.T. 10 produced a quick rise and fall in blood calcium. Bauer and Lindquist (89) reviewed the literature on the

effects of vitamin D on bone. They came to the conclusion that, although it cannot be regarded as a homeostatic agent in the strict sense of the word, as it is not a hormone, the production of which can be controlled by the needs of the organism; it appears to be involved in calcium homeostasis. In this connection they refer to Nicolaysen's (29) concept of an endogenous factor, by means of which the organism adapts absorption of calcium to the skeletal needs, but which does not function in the absence of vitamin D. Such a factor, the nature of which is obscure, could act to integrate the effects of the parathyroid hormone and vitamin D on homeostatic control of the calcium concentration in the blood plasma.

VI. ENDOCRINE CONTROL OF CALCIUM METABOLISM.

Some of the endocrine glands have profound effects upon the calcium level, whereas others have but little action. Thus insulin very probably increases the blood calcium, but this is secondary to its effect of lowering the inorganic phosphorus level. The thyroid has a considerable effect upon calcium excretion and in thyrotoxicosis there is an increased loss of calcium. There seems to be no unanimity about the level of calcium in this condition. Albright (90) considered it to be slightly raised, but Wade (91) found it to be lowered.

The action of estrogens on bone calcium is of great interest. It was first noted by Kyes and Potler in 1934 (92) that the female pigeon during the laying period developed an excess of spongy bone in the marrow cavity of the long bones. Where calcium was needed for egg shell formation the excess bone was rapidly removed. During this time, from preovulation until the eggs were laid, the blood calcium rose

to a high figure. This effect could be reproduced in male pigeons and also in mice (male and female) by the administration of estrogens (93). Male hormones had no such effects and the removal of pituitary or parathyroid did not prevent the hypercalcemia (94). The local application of estradiol to chips of bone implanted in the brain of litter mice was not followed by any bone change (95).

The concentration of blood calcium, phosphorus and phosphatase of mice does not change when estrogen is given. Urist et al (96) investigated the action of estrogens in other animals, and of those they tested young rats were the only ones found to be affected, but in a rather different way. Here the resorption of cartilage and new bone was prevented so that a very large spongiosa developed. In humans the long-continued administration of estrogen is followed by hyperossification (97) and defective bone growth and osteeosis could be found in both sexes in primary gonadal insufficiency.

Anderson and Oastler (98) in well-controlled experiments with rats found that hypophysectomy had no effect upon the blood calcium level. However, the effect of this organ on bone formation is of considerable interest. Hypophysectomy stops all endochondral bone sequences, the changes being like those in the ageing of normal animals (99, 100). On giving growth hormone to normal animals, the growth sequences persist and the bones are larger and longer (101). When growth hormone is given to hypophysectomised animals, endochondral ossification is at once stimulated and an almost normal histological picture is produced which is, however, continued for the whole experimental period so that skeletal

maturity, in the sense of closure of epiphyses, is not attained (102, 103). It may be that the growth hormone acts directly on the bone.*

Taylor and Cavon (105) first reported hypercalcemia in adrenalectomised rats. Tibbetts and Aub (106) and Leemska (107) also reported similar results. Hypercalcemia can also develop following adrenalectomy in animals which have first been parathyroidectomised, provided the serum calcium has not fallen excessively (105). Administration of adrenal cortical extracts (105) or of cortisone (108) to rabbits depressed the serum calcium. In Cushing's disease, however, normocalcemia appears to be the rule (109). Both Dent (110) and Connor *et al* (112) have reported that treatment with either cortisone or corticotropin was effective in depressing the high blood calcium level seen in sarcoidosis, idiopathic hypercalcemia of children, and vitamin D intoxication, but was not effective in hyper parathyroidism. The mechanism by which this is effected is not known.

The parathyroid glands have the most profound controlling influence upon calcium and phosphorus metabolism and have been widely studied. MacCallum (112) observed in 1909 that the convulsions and tetany reported by Vasali and Generali (113) following parathyroidectomy could be prevented

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* Using Ca^{45} as tracer, Urich and his co-workers (104) observed that the serum level of the isotope in hypophysectomised rats was twice as high as in normal animals. Whereas, the specific activity of the tibias of the hypophysectomised animals was lower than that of the controls. Administration of growth hormone given to hypophysectomised rats receiving Ca^{45} decreased the elevated serum isotope levels to normal, and increased the skeletal (tibial) uptake of Ca^{45}. In addition, hormone administration reduced to normal the increased faecal secretion of the isotope observed in hypophysectomised animals.

by the administration of calcium salts. These observations were confirmed by Hastings and Murray (114) who found that the hypocalcemia resulting from removal of the parathyroid glands could be temporarily corrected by the intravenous administration of calcium salts. In 1925 Collip (115) prepared an acid extract of parathyroid glands which was effective in restoring the normal plasma calcium level in parathyroidectomised animals, and noted the important function of these glands in the regulation of blood calcium level. The purified hormone, calcitonine, from the parathyroid extract which is responsible for the normal calcium balance in the body has been recently reported (116). It is now firmly established that the parathyroid glands play a decisive role in the homeostatic regulation of the calcium ion concentration of blood plasma. There is a direct correlation between the calcium ion levels in the body fluids and the state of activity of the parathyroid gland.

The two most important factors in regulating calcium are the vast reservoir of mineral in the bones (117) and the function of the parathyroid glands, which, as McLean (118) has pointed out serve as 'calciostats' to maintain the optimal level for this ion in the blood. Studies of radio calcium in man (119) indicate that 0.3% to 0.6% of the bone calcium is readily exchangeable and may provide a labile pool of readily available calcium. In addition, 0.2 gm. to 0.4 gm. of calcium per day may be deposited in bone by new bone formation, or by the increase in mineralization of newly formed osteones, while a corresponding amount of calcium may be released by bone resorption in remodeling, removal of trabeculae, or by erosion of Haversian canals. Rapid restoration of the normal level of plasma calcium has been demonstrated in a number of experiments

in which hypocalcemia had been induced by infusion of calcium depleted blood, oxalate, citrate or EDTA (120, 121, 122).

Although it is generally acknowledged that the primary effect of alterations in parathyroid hormone secretion is on the ionizable, diffusible calcium, these changes are not reflected in the cerebrospinal fluid (CSF) calcium. Cameron and Moorehouse (123) first reported that in parathyroidectomised dogs the CSF calcium level did not decrease as much as the serum calcium. Subsequently, Morgulis and Perley (124) confirming and extending these observations, found that the CSF calcium rose only slightly in dogs in which the serum calcium had risen considerably in response to parathyroid hormone administration. Merrit and Bauer (125) were unable to detect any change in the CSF calcium either in humans given the hormone or in hypoparathyroid patients, although in each instance the expected alterations in the serum calcium level were observed. More recently, Howard and his co-workers (126) corroborated these observations and pointed out that in hypercalcemias not due to hyperparathyroidism, an increase in CSF calcium was observed. One, therefore, wonders whether the calcium of the CSF is, in fact, freely diffusible in parathyroid dysfunction.

Another surprising finding was made by Munson and his colleagues (127) who found that, although parathyroidectomy of the lactating rat produced a decrease in the blood calcium level in milk flow, no change in the milk cälcium concentration occurred. There is, at present, no satisfactory explanation for any of these findings.

Reference should also be made to the recent reports concerning the effect of the parathyroid gland on calcium transport. Talmage and

Elliot (128) found that 2 - 4 hours after parathyroidectomy the absorption of radioactive calcium and strontium from ligated sections of rat intestine was reduced by 50%. Rasmussin (129) found that inverted sacs of small intestine from parathyroidectomised rats showed a decreased ability to develop and maintain a concentration difference between serosal and mucosal bathing fluids. Finally, Reaven et al (130) had reported that parathyroid extracts inhibited the in vitro uptake of calcium by rat abdominal musculature. It was suggested that the hormone regulates the movement of calcium between extracellular and intracellular fluids.

VII. CALCIUM AND MUSCLE CONTRACTION.

A. Smooth muscles: Calcium has long been proposed as the link between membrane depolarization and contraction (131, 132). Of all the physiologic ions that have been injected in small quantities, calcium alone causes contraction (133). If the presence of free calcium ion is primarily responsible for contraction of muscle protoplasm, then it is logical to assume that in general, various types of chemical and electrical stimulation would cause a release of calcium ion into the interior of the muscle cells. This has been observed by Woodward (134) and confirmed by Shanes and Bianchi (135). Shanes and Bianchi (135) also demonstrated the release of Ca^{45} during a brief tetanic stimulation from frog sartorious muscle that has been previously soaked in Ca^{45} Ringer's solution. Potassium contractures, both isotonic and isometric, increase calcium outflux. The increased outflux during tetanic stimulation is not sustained and the minimum calcium released per twitch is about the same as the amount taken up per twitch.

Potassium contracture results in a rapid release of calcium, which is about double the base line rate even after 10 minutes (135). The increased influx and outflux of calcium in frog sartorious muscle observed with tetanic stimulation or potassium contracture may reflect the same basic process, such as freeing of calcium from the surface, supported by the rapid release of calcium during tetanic stimulation. Two other possible explanations for increased influx and outflux are:

1. a spatial separation in which different sites of the membrane involve calcium influx and outflux, and,
2. a temporal separation of the two fluxes.

From the foregoing, it is evident that calcium influx into muscle fiber is related to mechanical activity in two ways. The enhanced twitch height and contracture in nitrate Ringer's solution is correlated with a larger influx of calcium, and there is a temporal relationship between the duration of increased calcium influx and mechanical activity. Potassium contractures and a high rate of calcium influx are transitory in phasic muscles, while in slow fibers contratures are sustained as is the high rate of calcium influx. The manner in which calcium brings about activation is still unknown, although from experiments on model systems there appears to be two possible modes of action. One would be the inhibition of the relaxing factor system, thus allowing contraction to take place, with relaxation occurring as the ionized calcium is removed; the other would be by a direct action of calcium on actomysin.

B. Cardiac muscle: The importance of calcium in the contraction of heart muscle has been known since the experiments of Ringer (136).

Early work (137) focused primarily on the dependence of the strength of contraction on the concentration of calcium ions in bathing solution. More recently an antagonism between sodium and calcium ions at the cell surface has been inferred from the observation that the effects of a decrease in extra-cellular sodium concentration and an increase in extra-cellular calcium concentration on twitch tension are similar (138). The observation that withdrawal of calcium ions from the bathing solution caused rapid disappearance of mechanical but not of electrical activity of isolated heart muscle implicated the calcium ion as the excitation-contraction link (139).

In a series of studies on frog ventricular strips, Niedergerke and Liittan (140) showed that changes in external sodium and calcium concentrations very rapidly altered the characteristics of potassium-induced contracture and that these changes in contracture tension could be produced even after the initial potassium depolarization was complete.

Weidman (141) demonstrated in turtle ventricle that a sudden increase in the concentration of calcium in the extra-cellular space during the initial stages of a twitch produced a more rapid rate of tension development and a greater peak tension than occurred at lower calcium concentration. In addition, the increase in calcium concentration was accompanied by a shortening of the action potential. A hypothesis that incorporates these data with the observed correlation between the rate of calcium influx during a contraction and the size of contraction has been proposed as follows: the calcium that enters the cell with contraction comes from superficial sites and from extracellular fluid; during depolarization it passes through the same superficial sites to which calcium was bound in resting stage (141).

VIII. CALCIUM AND ENZYMES.

Calcium participates in a number of enzyme systems by forming ionic complexes with the enzyme and/or substrate. Almost all enzymes which catalyze group transfer require activation by divalent metal ions, which in turn form essentially ionic complexes. When the group transfer is a substituted phosphoric acid, the usual activator is an alkali earth metal (142). The following table illustrates the participation of calcium ion in a number of enzymatic reactions both as an activator and as an inhibitor of enzymes.

ENZYME	SOURCE	COFACTOR
A. As enzyme activator		
Adenylate Kinase (crystalline enzyme)	Rabbit skeletal muscle	Ca^{2+}, Mg^{2+}.
ATP ase (myosin) (crystalline enzyme)	Rabbit skeletal muscle	Ca^{2+}
Lipase	Hog pancreas	Ca^{2+}
Phospholipase A	Venom of crotalus terrifieus	Ca^{2+}
Phospholipase C	Clostridium welchii	Ca^{2+}
Thromboplastin	Fresh beef lung	Ca^{2+}
Aldehyde delydrogenase	Baker's yeast	Ca^{2+}, Mg^{2+}, TPN
B. As enzyme inhibitors:		
Glutamic synthetase	green peas	Ca^{2+}
Firefly luciferase	Photinus pyralis	Ca^{2+}

ENZYME	SOURCE	COFACTOR
B. As enzyme inhibitors (continued).		
Flavokinase	yeast	Ca^{2+}
5-Nucleotidase	Bull semem	Ca^{2+}
Citritase	E. Coli A. Aerogenes	

This table has been reproduced from Comar and Bronnes, Mineral Metabolism V.l. Pt. B (142).

METHODS

A. Animals:

All animals used throughout the investigation were adult male albino rats of the Sprague-Dawley strain, weighing from 150 to 200 gm. They were kept under identical conditions in the same animal room and maintained on Purina fox chow and water *ad libitum*.

Rats of the same strain hypophysectomised by the transpharyngeal route were obtained from the Hormone Assay Laboratories Inc., Chicago, Illinois, U.S.A. They were maintained on a diet of oranges, carrots, brown bread and water.

Adrenalectomy was performed by the dorsal route while the animal was under ether anesthesia. Adrenalectomised rats were kept on the normal Purina fox chow diet and were given one percent sodium chloride solution *ad libitum*. Following operation, adrenalectomised animals were observed for a 48-hour recovery period before they received the injection of hormone.

Thyroparathyroidectomy was performed in the laboratory using the procedure described by Farris and Grifith (143). Following operation blood samples were collected from the tail of the rat for two to three days and the calcium concentration was measured. A fall of calcium concentration to a level of 70% of normal animals at the end of three days was used as a criteria of the success of the thyroparathyroidectomy. The thyroparathyroidectomised animals were kept on Purina fox chow diet and water *ad libitum*.

B. Injections:

Adrenocorticotrophic hormone (ACTH) was administered intraperitoneally to both normal and hypophysectomised rats. The dose of ACTH was 4 mg per 100 gm. body weight administered as a single dose four hours before the intravenous injection of radio calcium.

Growth hormone (STH) was administered by intraperitoneal injection to both intact and hypophysectomised rats. The dose of STH was 0.2 mg per day for a period of 14 days.

Desoxycorticosterone acetate (DCA) injection solution was made in sesame oil and was administered intraperitoneally to both intact and adrenalectomised rats. The amount of DCA injected was 6 mg as a single dose given 24 hours before the administration of radio calcium.

Thyroid extract (TE) was administered as an intraperitoneal injection to both intact and thyroparathyroidectomised rats. A dose of 0.1 mg per 100 gm body weight was administered for a period of 7 days. This dose was calculated from the U. S. P. standard dose.

Parathyroid extract (PTE) was also administered intraperitoneally to both intact and thyroparathyroidectomised rats. A dose of 0.2 mg. per 100 gm. body weight was administered for a period of 7 days.

Ca^{45} was obtained from the Oak Ridge Laboratories and was made available through the Atomic Energy of Canada Limited. The Ca^{45} was in the form of $CaCl_2$ solution in HCl and was a specially irradiated sample having a specific activity of approximately 473 mcs/gm

A diluted solution of stock Ca^{45} solution was injected intravenously via the external jugular vein while the animal was under either

anesthesia. The amount used was 20 μc per animal and the volume was never greater than 0.25 ml. The calcium ion concentration never exceeded 100 μg/ml. The dilution was made using demineralized distilled water. Known dilutions of the injection solution were used to prepare standards.

C. Tissue Samples:

The rats were sacrificed by decapitation and blood samples were collected by inverting the body over a micro-beaker. A vertical incision was made up the abdomen and the adrenals removed and cleaned of adhering adipose tissue, placed in a 5 ml Fernback flask and weighed immediately. Samples of liver and heart tissue weighing between 50-100 mg were similarly excised, blotted to remove excess blood, placed in the flask and weighed. The prostate was separated into the dorsolateral and ventral portions.

The Fernback flasks containing the tissue samples were then heated at 90°C for 30 minutes following the addition of 0.5 ml of two parts concentrated nitric acid and one part perchloric acid. The temperature was gradually increased to 200°C over a 2 hour period and the samples were allowed to ash overnight.

D. Chemical Analysis of Calcium:

The method selected for this investigation was the spectrophotometric method reported by Reynolds and Linde (144). The ashed tissue samples were dissolved in 1.0 ml 0.02N HCl and transferred to 3 ml centrifuge tubes with two subsequent 0.5 ml washings with demineralized

distilled water. To each tube 0.5 ml of 2% stannic chloride in ethanol was then added and the tube placed in a 70°C water bath for 30 minutes. The tubes were then cooled and centrifuged.

Portions (1.5 ml) of the supernatants were pipetted to 5 ml volumetric flasks and 2 ml of 25% ethanol in water (v/v) were added. Sufficient amounts of IN NaOH containing 2.5% KCN were added to obtain a PH of 12.0 ± 0.5 and subsequently 0.5 ml of piperidine buffer, PH 12, was added to each flask. Immediately prior to reading in the spectrophotometer, 0.5 ml of 5×10^{-4} M murexide was added and optical measurements were carried out within 5 minutes after addition of the dye.

A Beckman DU spectrophotometer was used to determine the absorption of calcium-murexide at 495 mμ. For each set of analyses a reagent blank was carried through the entire procedure. Calcium standards were carried through from the beginning of the stannic chloride precipitation step and standard curves were determined each time with known amounts of calcium. These were linear over the range 0 to 6 mg calcium per sample (Fig. I).

E. Determination of Radioactivity:

After the sample had been analyzed for calcium colorimetrically according to the method described above, the contents of the 5 ml volumetric flask were transferred quantitatively with two subsequent washings with 0.5 ml 95% alcohol to a polyethylene plastic tube with a nickel-steel cup forming the bottom (145). A standard amount of carrier calcium (0.5 mg) was added. Then 1 ml of concentrated murexide solution and 1 ml 95% alcohol were added to the tube. After standing one-half hour the tube

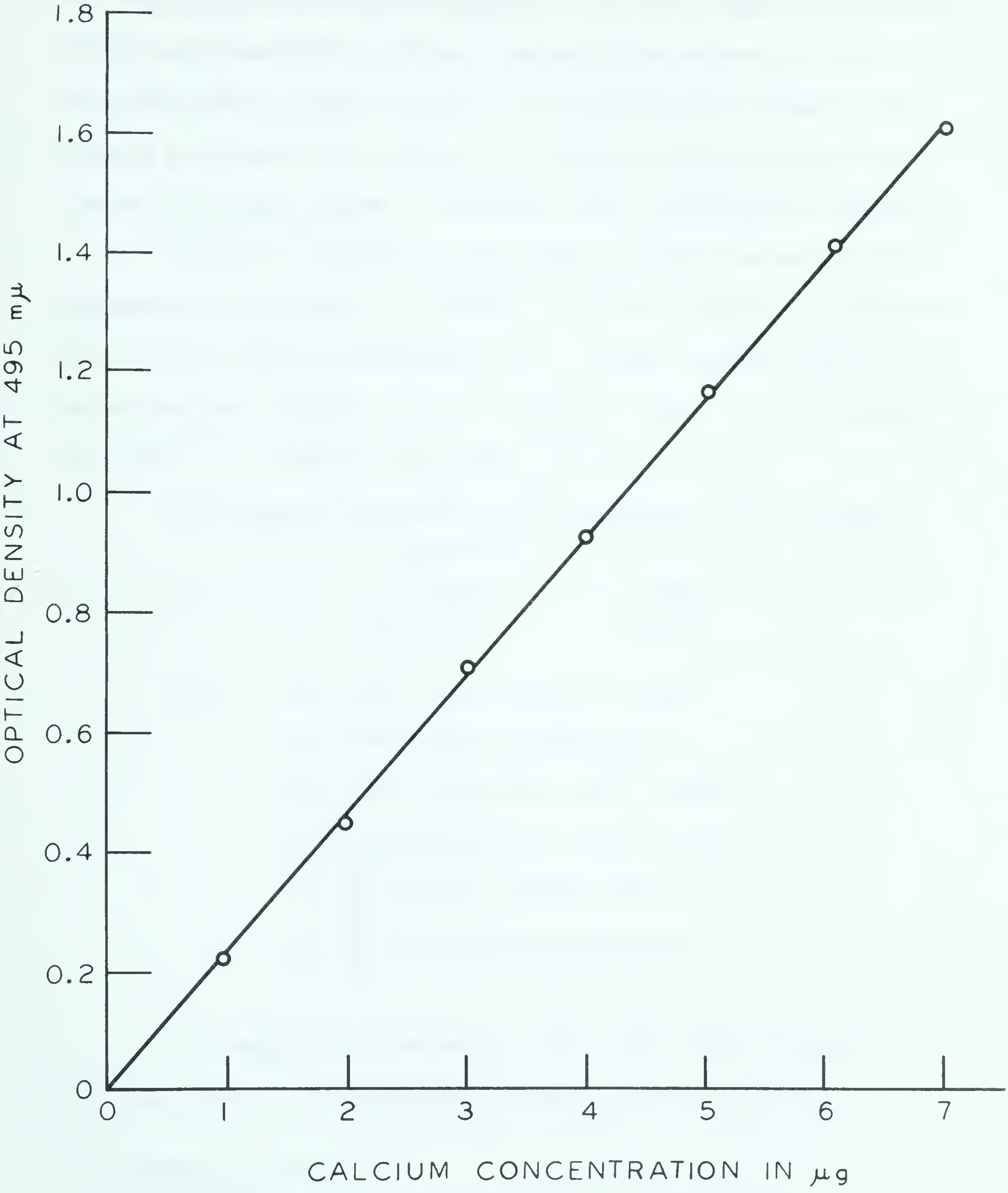


Fig. I. Relationship between optical density at 495 mμ and concentration of calcium.

was centrifuged with care being taken to prevent leakage, and the supernatant was drawn off by suction. The cups were removed from the tubes and dried under an infra red lamp. The radioactivity present in the samples was measured by counting in a Nuclear Chicago gas flow G.M. counter with Mylar window. All samples were corrected for background.

In order to correct for variations in the injected dose from experiment to experiment, a standard of Ca^{45} was prepared at the same time as each series of injections, with the same syringe used for the injections, and diluted in 100 ml volumetric flask. A 20λ aliquot was placed on a planchet and counted for radioactivity.

The standard counting error was determined by the formula:

$$n_s - n_b = \pm \sqrt{\left(\frac{\sqrt{N_s}}{t_s}\right)^2 + \left(\frac{\sqrt{N_b}}{t_b}\right)^2}$$

where, N_s = total sample count recorded

t_s = total sample counting time

N_b = total background count recorded

t_b = total background counting time.

$n_s = \frac{N_s}{t_s}$ = sample counting rate.

$n_b = \frac{N_b}{t_b}$ = background counting rate

All samples were counted for 5000 counts using automatic time interval printer. The percentage standard error was $\pm$ 2.01%.

F. Definition of Terms:

Calcium concentration was expressed as μg of calcium per gm wet weight of tissue.

The specific activity of calcium in a tissue was defined as the counts per minute per μg of calcium in the tissue.

$$\text{Specific activity} = \frac{\text{Net sample corrected C/m}}{\mu\text{g calcium in the sample}}$$

G. Statistical Analysis:

For significance in the statistical analysis, the calculation for confidence limits of the mean was done according to the following formula from Kenny and Keeping (146).

$$t = \frac{(\bar{X} - \mu) \quad n^{\frac{1}{2}}}{S}$$

where, $\bar{X}$ = Mean.

t = student's t.

$n = N-1$

where N = the sample size

(N = number of observations).

S = Standard deviation of the Mean.

μ = 95% confidence limit of the Mean.

This formula is used to establish the 95% confidence limits and where significance is indicated it therefore means confidence at the .05 level of probability.

RESULTS

Preliminary to the experimental work was the necessity to establish a satisfactory method for the determination of the calcium ion and the Ca^{45} activity in the same sample. A satisfactory method combining the analytical procedure for the colorimetric determination of the calcium ion in tissue samples using murexide, as reported by Reynolds and Richard (144), with a specially devised procedure for the preparation of samples for counting, as described in methods under Section E, (page 25) was established. This method has been published (147).

In this method the addition of ethanol to a concentration above 25% resulted in the precipitation of calcium-murexide at pH 12 ± 0.2. It was found that 2 ml of 95% alcohol precipitated all the calcium-murexide quantitatively. That the complete precipitation was obtained was examined by the addition of a known amount of radioactive calcium before the precipitation step, and by subsequent counting of a portion of the concentrated supernatant. The amount of carrier calcium required for a well distributed standard sample was found to be 0.5 mg. The amount of murexide added was a calculated equivalent amount, sufficient to precipitate out all the calcium present in the sample. A study of the percentage recovery in a series of test samples was carried out. Known amounts of standard Ca^{45} were added to different samples, having different amounts of carrier calcium, and these samples were counted for radioactivity. The results of this study are presented in Table I which is reprinted from the Canadian Pharmaceutical Journal, Science Section (147).

The fraction counted (N/No) was calculated by dividing the individual counts (N) of samples by the arithmetic mean of 20 samples of known amount of standard Ca^{45}.

TABLE I

PERCENTAGE RECOVERY IN TEST SAMPLES

Added carrier calcium	Murexide Added	* Fraction counted (N/N_o)	% Recovery	Coefficient of variability	Density Thickness of the Sample
0.01 mg.	0.02 ml	0.79 ± .080	79 ± 8.0	10.13%	0.07 mg/cm^2
0.05 mg.	0.10 ml	.77 ± .074	77 ± 7.4	9.60%	.41 mg/cm^2
0.10 mg.	.20 ml	.79 ± .071	79 ± 7.1	9.00%	.80 mg/cm^2
0.25 mg.	.50 ml	.88 ± .063	88 ± 6.3	7.16%	1.80 mg/cm^2
0.50 mg.	1.00 ml	.90 ± .040	90 ± 4.0	4.44%	3.54 mg/cm^2
0.75 mg.	1.50 ml	.89 ± .040	89 ± 4.0	4.48%	5.40 mg/cm^2
1.00 mg.	2.00 ml	.88 ± .040	89 ± 4.0	4.48%	7.16 mg/cm^2
** IN TISSUE SAMPLES.					
0.50 mg.	1.00 ml	.87 ± .031	87 ± 3.10	3.55%	--

* Mean of five samples

** Mean of ten samples

Arithmetic mean	=	1868 c/m (N_o)
S.D.	=	± 64
Probable % of error	=	2.300%

The same amount of Ca^{45} was added to each test sample.

The thickness of the sample was calculated by dividing the area of the planchet by the weight of each sample (assuming the distribution to be uniform). Figure II (reprinted from C.Ph.J.) describes the relation between the fraction counted and the thickness of the sample. From the graph it is obvious that the thickness of sample between 3 mg/cm^2 and 4 mg/cm^2 will give a satisfactory percentage recovery with a minimum coefficient of variability. This approximation corresponds to the addition of 0.4-0.6 mg of carrier calcium, 0.5 mg being chosen as the median of the two.

The percentage recovery of Ca^{45} added to the adrenal glands was determined in 10 different samples. Recoveries ranged from 84 to 90% with the mean and standard deviation being 87.1 ± 3.1%.

An evaluation of the time after the administration of Ca^{45} to determine the most suitable time for satisfactory measurement for the program which had been planned was also considered necessary.

It was considered desirable to keep the dose of Ca^{45}, together with the small amount of carrier calcium, at as low a level as possible. The decision was to administer 20μc of Ca^{45} (see page 24). The percentage of the injected dose found per gm of tissue at the different time intervals studied is presented in Figure III. Each of the points on the curves in Figure III are the mean of five individual experiments.

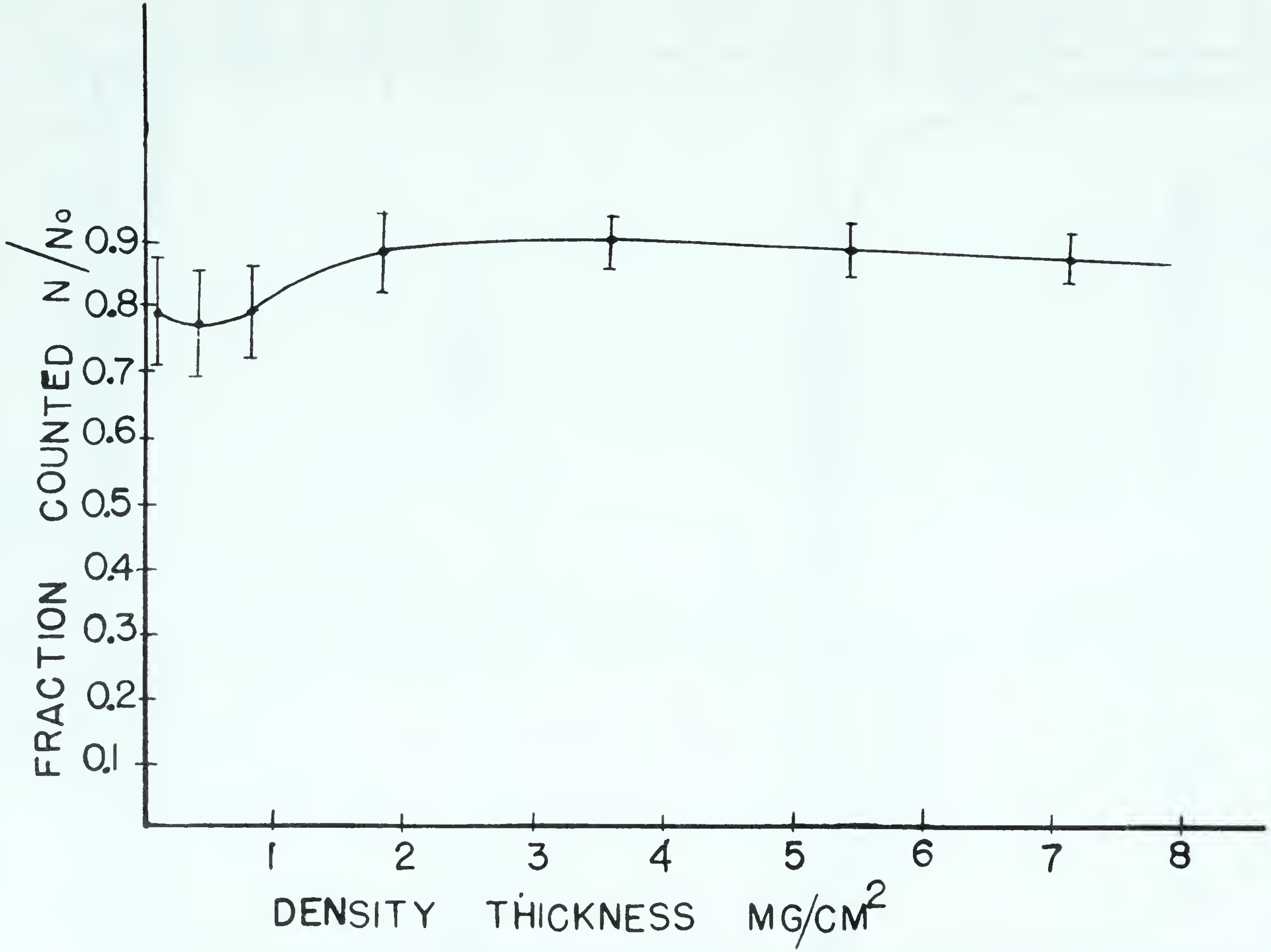
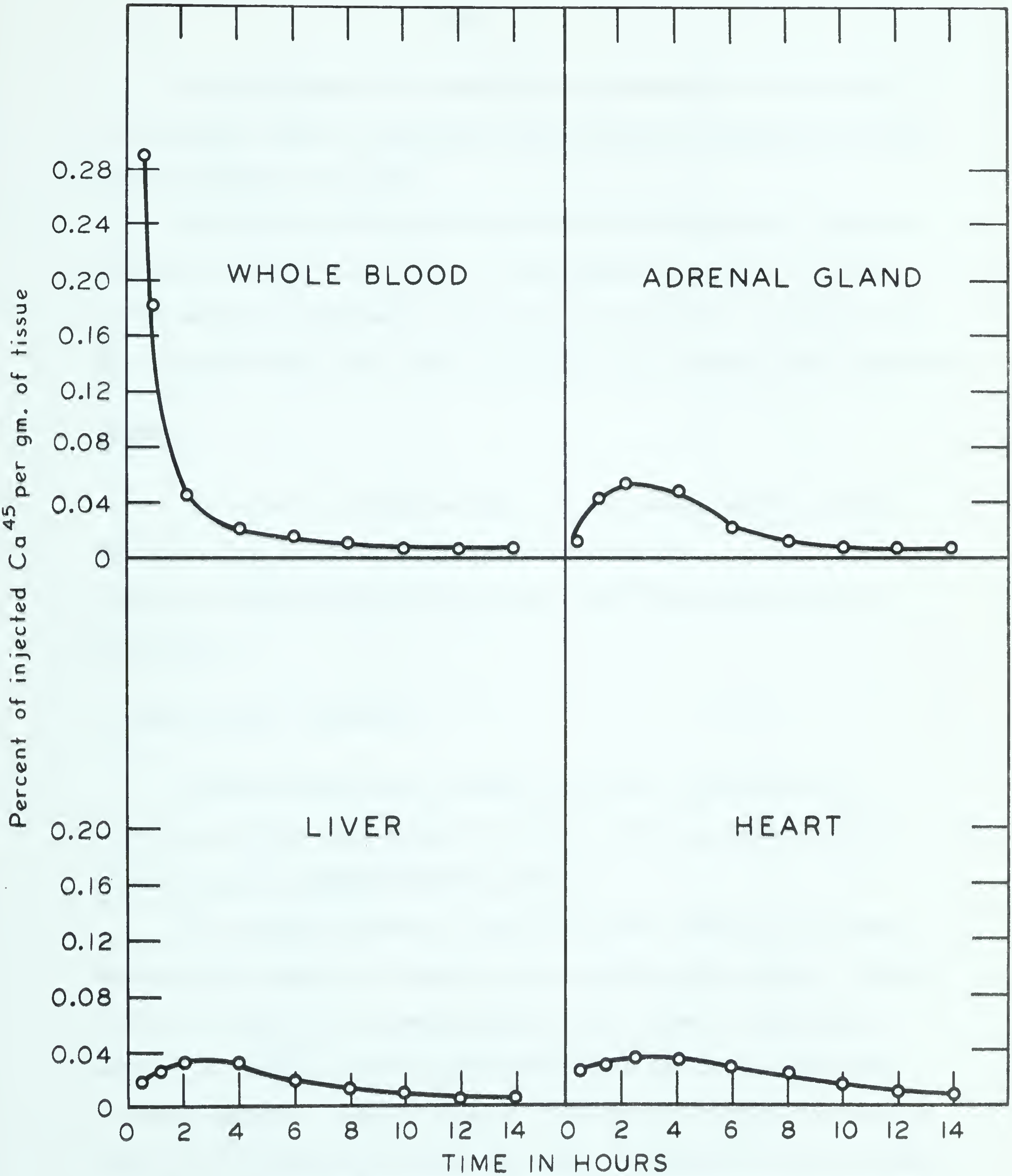


FIGURE II RELATION BETWEEN THICKNESS OF SAMPLE AND FRACTION COUNTED (N/No)

Each point represents the mean of five samples
The vertical lines represent the standard deviation of each point.



(each point represents the mean of five individual experiments)

Fig. III. Relationship between percent of injected Ca^{45} per gm. of tissue and time in hours.

It was concluded that satisfactory measurements in the tissues to be studied could be made using a two hour period following the intravenous injection of the Ca^{45}.

The results of the experimental work are presented in separate sections according to the series of experiments undertaken. For each of the series of experiments the results are presented in tabular form. The data from which these tables are derived are included in the Appendices.

SERIES I

The effect of hypophysectomy, and the administration of ACTH and STH in both normal and hypophysectomised animals, on the concentration of calcium and the distribution of Ca^{45} has been studied in this series.

A. Whole Blood: (Table II)

Hypophysectomy did not affect the calcium concentration of blood, nor did the administration of ACTH or STH when administered to either normal or hypophysectomised animals.

The specific activity of the blood almost doubled in the hypophysectomised animals as compared to that in the normal animals. Administration of ACTH to the hypophysectomised rats caused a significant decrease in specific activity which approached but did not equal that of normal animals. Administration of STH however, returned the elevated level of Ca^{45} activity to the normal level. ACTH given to normal animals resulted in an increase in Ca^{45} activity in blood which was not significant. STH apparently caused no change in activity when administered to normal animals.

TABLE II

Whole Blood - Effect of Hypophysectomy and Administration of ACTH and STH on Calcium Concentration and Ca-45 Distribution

Groups of Animals	No. of Animals	Calcium Concentration μg/ gm Wet Weight	Sig.*	Specific Activity	Sig.*
		$\bar{X}$ ± SEM		$\bar{X}$ ± SEM	
Normal Control	20	60.92 ± 2.94		174.89 ± 14.94	
Normal + ACTH	14	59.67 ± 2.10		258.07 ± 10.76	S
Normal + STH	20	61.12 ± 1.45		185.73 ± 4.25	
Hypophysectomised	20	59.80 ± 2.19		464.14 ± 19.60	S
Hypophysectomised + ACTH	16	67.00 ± 4.32		244.37 ± 15.21	
Hypophysectomised + STH	20	60.38 ± 1.52		187.56 ± 8.54	

* Significance calculated using the formula $t = \frac{(\bar{X} - \mu)\, n^{\frac{1}{2}}}{S}$

B. Adrenal Gland: (Table III).

Hypophysectomy caused a significant increase in calcium concentration in the gland, which returned approximately to the normal level under the influence of injected ACTH. Administration of STH had no effect on the elevated calcium concentration in the adrenal gland of these hypophysectomised animals. When ACTH was administered to normal animals a significant increase in the concentration of calcium was observed. An even greater increase was observed when STH was administered.

The specific activity of the gland decreased considerably as a result of hypophysectomy. No significant effect was observed when ACTH was administered to hypophysectomised rats. Administration of STH markedly increased the lowered activity observed in the hypophysectomised rats to about the level apparent in normal animals. Normal animals when administered a similar dose of STH showed no observable difference in specific activity to that in normal rats. Administration of ACTH however, resulted in a very significant decrease in the specific activity in the adrenals of normal rats.

C. Dorsolateral Prostate: (Table IV)

The calcium concentration in the dorsolateral prostate was significantly decreased after hypophysectomy. Administration of both ACTH and STH to the hypophysectomised rats showed the correction effect. When ACTH was administered to the normal animals the calcium concentration in the tissue decreased significantly. Administration of STH to the

TABLE III

Adrenal Gland - Effect of Hypophysectomy and Administration of ACTH and STH on Calcium Concentration and Ca-45 Distribution

Group of Animals	No. of Animals	Calcium Concentration μg/ gm Wet Weight	Sig.	Specific Activity	Sig.
		$\bar{X} \pm$ SEM		$\bar{X} \pm$ SEM	
Normal Control	20	60.73 ± 3.18		1009.83 ± 62.85	
Normal + ACTH	14	73.38 ± 2.51		505.47 ± 14.98	
Normal + STH	20	97.05 ± 2.45	S	1001.53 ± 29.65	S
Hypophysectomised	20	105.51 ± 3.10	S	751.37 ± 34.29	
Hypophysectomised + ACTH	16	74.79 ± 3.67		678.05 ± 45.86	S
Hypophysectomised + STH	20	104.20 ± 3.64		932.63 ± 29.16	

TABLE IV

Dorsolateral Prostate - Effect of Hypophysectomy and Administration of ACTH and STH on Calcium Concentration and Ca-45 Distribution

Group of Animals	No. of Animals	Calcium Concentration μg/ gm Wet Weight	Sig.	Specific Activity	Sig.
		$\bar{X}$ ± SEM		$\bar{X}$ ± SEM	
Normal Control	20	97.10 ± 6.18		219.90 ± 17.17	
Normal + ACTH	14	62.70 ± 2.03		523.24 ± 18.02	
Normal + STH	20	109.45 ± 3.93	S	306.07 ± 9.94	S
Hypophysectomised	20	63.64 ± 3.32		714.48 ± 39.62	
Hypophysectomised + ACTH	14	97.30 ± 5.00	S	577.57 ± 45.53	S
Hypophysectomised + STH	13	94.43 ± 4.07		321.66 ± 15.02	

normal animals did not produce any significant change.

The specific activity in the dorsolateral prostate of the hypophysectomised rats was increased significantly to that in the normal rats. Administration of ACTH to the hypophysectomised rats did not produce any significant effect. Administration of STH to the hypophysectomised rats caused a significant decrease in specific activity which approached but did not equal that in the normal animals. Normal animals when administered a similar dose of ACTH showed a significant increase in specific activity. Administration of STH to the normal animals also showed a significant increase in specific activity.

D. Ventral Prostate: (Table V).

Hypophysectomy caused a significant increase in calcium concentration in the ventral prostate which returned to the normal level under the influence of both ACTH and STH administration. Administration of ACTH to the normal animals produced a significant increase in calcium concentration to that in the normal animals. STH apparently showed no change in calcium concentration when administered to the normal animal.

The specific activity in the ventral prostate was increased significantly after hypophysectomy. Administration of ACTH to the hypophysectomised animals did not produce any change in specific activity. When STH was administered to the hypophysectomised rats the specific activity in the tissue showed no significant difference from that in the normal animals. Normal animals when administered ACTH showed no

TABLE V

Ventral Prostate - Effect of Hypophysectomy and Administration of ACTH and STH on Calcium Concentration and Ca-45 Distribution

Group of Animals	No. of Animals	Calcium Concentration μg/ gm Wet Weight	Sig.	Specific Activity	Sig.
		$\bar{X} \pm$ SEM		$\bar{X} \pm$ SEM	
Normal Control	20	40.63 ± 2.99		384.13 ± 20.98	
Normal + ACTH	14	53.88 ± 2.07		307.27 ± 15.59	
Normal + STH	20	47.40 ± 1.82	S	469.62 ± 12.02	S
Hypophysectomised	20	60.72 ± 2.21		517.28 ± 34.02	
Hypophysectomised + ACTH	14	40.60 ± 3.68	S	554.46 ± 45.47	
Hypophysectomised + STH	13	46.86 ± 1.81		389.85 ± 21.94	

significant change from that in the normal animals. Administration of STH to the normal animals, however, produced a significant increase in specific activity from that in the normal animals.

E. Liver and Heart: (Tables VI and VII)

Neither the calcium concentration nor the specific activity in these tissues showed any significant difference in the various groups of animals studied.

TABLE VI

<u>Liver</u> - Effect of Hypophysectomy and Administration of ACTH and STH on Calcium Concentration and Ca-45 Distribution

Group of Animals	No. of Animals	Calcium Concentration μg/ gm Wet Weight	Sig.	Specific Activity	Sig.
		$\bar{X} \pm$ SEM		$\bar{X} \pm$ SEM	
Normal Control	14	37.60 ± 3.17		346.81 ± 32.99	
Normal + ACTH	11	38.70 ± 1.81		298.90 ± 8.51	
Normal + STH	12	43.82 ± 1.81		364.99 ± 17.95	
Hypophysectomised	12	44.16 ± 1.52		406.12 ± 24.47	
Hypophysectomised + ACTH	12	50.75 ± 4.73		399.81 ± 45.93	
Hypophysectomised + STH	13	41.52 ± 1.32		378.21 ± 28.29	

TABLE VII

Heart - Effect of Hypophysectomy and Administration of ACTH and STH on Calcium Concentration and Ca-45 Distribution

Group of Animals	No. of Animals	Calcium Concentration μg/gm Wet Weight	Sig.	Specific Activity	Sig.
		$\bar{X} \pm$ SEM		$\bar{X} \pm$ SEM	
Normal Control	14	46.65 ± 5.35		297.14 ± 21.53	
Normal + ACTH	11	44.62 ± 3.01		263.60 ± 12.46	
Normal + STH	12	46.43 ± 2.67		401.20 ± 27.62	
Hypophysectomised	12	58.64 ± 3.66		369.50 ± 50.68	
Hypophysectomised + ACTH	12	54.12 ± 4.58		403.24 ± 31.56	
Hypophysectomised + STH	13	47.10 ± 2.10		312.81 ± 15.13	

SERIES II

The effect of adrenalectomy and the administration of DCA in both normal and adrenalectomised animals on the concentration of calcium and the distribution of Ca^{45} has been studied in this series.

A. Whole Blood: (Table VIII).

Adrenalectomy caused a significant increase in calcium concentration of blood. Administration of DCA to the adrenalectomised rats caused a significant decrease in calcium concentration which appeared to completely prevent the effect of adrenalectomy. Administration of DCA to the normal rats did not produce any significant change in calcium concentration of blood.

The specific activity of the blood in the adrenalectomised rats was increased significantly over that in the normal rats. Administration of DCA to the adrenalectomised rats caused a significant decrease in specific activity which approached but did not equal that of normal animals. Normal animals when administered DCA also showed a significant increase in specific activity.

B. Dorsolateral Prostate: (Table IX)

The calcium concentration in the dorsolateral prostate was significantly decreased after adrenalectomy. Administration of DCA to the adrenalectomised rats did not produce any significant effect. Normal animals when administered DCA showed a significant decrease in calcium concentration to that in normal animals.

TABLE VIII

<u>Whole Blood</u> - Effect of Adrenalectomy and Administration of DCA on

Calcium Concentration and Ca-45 Distribution

Group of Animals	No. of Animals	Calcium Concentration μg/gm Wet Weight	Sig.	Specific Activity	Sig.
		$\bar{X} \pm$ SEM		$\bar{X} \pm$ SEM	
Normal Control	20	60.92 ± 2.94		174.89 ± 14.94	
Normal + DCA	20	61.11 ± 1.25	S	368.77 ± 9.22	S
Adrenalectomised	14	79.81 ± 4.41		505.36 ± 4.41	
Adrenalectomised + DCA	14	61.37 ± 2.50	S	281.03 ± 16.57	S

TABLE IX

Dorsolateral Prostate - Effect of Adrenalectomy and Administration of DCA on Calcium Concentration and Ca-45 Distribution

Group of Animals	No. of Animals	Calcium Concentration $\mu g/gm$ Wet Weight	Sig.	Specific Activity	Sig.
		$\bar{X} \pm$ SEM		$\bar{X} \pm$ SEM	
Normal Control	20	97.10 ± 6.18		219.90 ± 17.17	
Normal + DCA	20	59.39 ± 2.14	S	552.58 ± 22.14	S
Adrenalectomised	14	50.44 ± 3.08		343.90 ± 16.16	
Adrenalectomised + DCA	14	51.56 ± 1.99		334.83 ± 12.25	

The specific activity in the dorsolateral prostate of the adrenalectomised rats increased significantly to that in the normal animals. Administration of DCA to the adrenalectomised rats did not produce any significant effect. Normal animals when administered DCA showed a significant increase in specific activity to that in the normal animals.

C. Ventral Prostate: (Table X)

Adrenalectomy caused no change in calcium concentration in the ventral prostate of the different groups of animals.

The specific activity in the ventral prostate of the adrenalectomised rats decreased significantly to that in normal animals. Administration of DCA to the adrenalectomised rats did not produce any significant difference to that in the adrenalectomised rats. However, there was a slight increase in specific activity when DCA was administered to the normal animals but this was not significant.

D. Liver and Heart: (Table XI and XII)

Adrenalectomy did not produce any effect in calcium concentration in the liver and heart. Administration of DCA to the adrenalectomised rats showed no effect. Administration of DCA to the normal rats showed a significant increase in calcium concentration in the liver to that in the normal animals. No change in calcium concentration was observed in the heart tissue when DCA was administered to the normal rats.

There was no significant difference in specific activity of the liver and heart tissue in the different groups of animals.

TABLE X

Ventral Prostate - Effect of Adrenalectomy and Administration of DCA on

Calcium Concentration and Ca-45 Distribution

Group of animals	No. of Animals	Calcium Concentration $\mu g/gm$ Wet Weight	Sig.	Specific Activity	Sig.
		$\bar{X} \pm$ SEM		$\bar{X} \pm$ SEM	
Normal Control	20	40.63 ± 2.99		384.13 ± 20.98	-
Normal + DCA	20	51.36 ± 2.26		469.13 ± 19.82	S
Adrenalectomised	14	39.46 ± 1.84		240.48 ± 14.13	-
Adrenalectomised + DCA	14	43.68 ± 1.90		281.26 ± 10.61	

TABLE XII

<u>Liver</u> - Effect of Adrenalectomy and Administration of DCA on Calcium Concentration and Ca 45 Distribution

Group of Animals	No. of Animals	Calcium Concentration μg/gm Wet Weight	Sig.	Specific Activity	Sig.
		$\bar{X} \pm$ SEM		$\bar{X} \pm$ SEM	
Normal Control	20	37.60 ± 3.17	S	346.81 ± 32.99	
Normal + DCA	20	46.59 ± 1.74		328.49 ± 11.24	
Adrenalectomised	14	36.49 ± 1.56		324.83 ± 20.52	
Adrenalectomised + DCA	14	37.75 ± 1.37		291.86 ± 12.24	

TABLE XII

Heard - Effect of Adrenalectomy and Administration of DCA on Calcium Concentration and Ca-45 Distribution

Group of Animals	No. of Animals	Calcium Concentration μg/gm Wet Weight	Sig.	Specific Activity	Sig.
		$\bar{X} \pm$ SEM		$\bar{X} \pm$ SEM	
Normal Control	20	46.65 ± 5.35		297.14 ± 21.53	
Normal + DCA	20	40.29 ± 2.05		353.96 ± 20.46	
Adrenalectomised	14	37.88 ± 1.51		302.12 ± 13.37	
Adrenalectomised + DCA	14	40.06 ± 1.12		296.97 ± 12.21	

SERIES III

The effect of thyroparathyroidectomy and the administration of PTE and TE in both normal and thyroparathyroidectomised animals on the concentration of calcium and the distribution of Ca^{45} has been studied in this series.

A. Whole Blood: (Table XIII)

Thyroparathyroidectomy caused a significant decrease in calcium concentration in blood. Administration of thyroid hormone showed no effect on the calcium concentration of blood. Parathyroid extract, however, showed the correction effect.

There was no significant change in specific activity in the thyroparathyroidectomised rats. Administration of TE or PTE caused no effect on thyroparathyroidectomised rats. However, administration of PTE to normal rats showed a significant increase in specific activity.

B. Adrenal Glands: (Table XIV)

Thyroparathyroidectomy caused a decreased calcium concentration in the adrenal gland. Administration of TE showed no effect. Thyroparathyroidectomised rats when administered PTE showed what appeared to be a recovery to normal.

The specific activity in the adrenal gland of thyroparathyroidectomised rats was the same as that of normal rats. TE or PTE extract administration showed no effect. However, there was a significant

TABLE XIII

<u>Whole Blood</u> - Effect of Thyro-Parathyroidectomy and Administration of TE and PTE on Calcium Concentration and Ca-45 Distribution

Group of Animals	No. of Animals	Calcium Concentration μg/gm Wet Weight	Sig.	Specific Activity	Sig.
		$\bar{X}$ ± SEM		$\bar{X}$ ± SEM	
Normal Control	20	60.92 ± 2.94		174.89 ± 14.94	
Normal + TE	8	60.16 ± 1.66	S	156.26 ± 8.94	S
Normal + PTE	8	65.36 ± 1.73		424.58 ± 24.57	
Thyro-parathyroidectomised	8	43.75 ± 1.39		288.58 ± 23.19	
Thyro-parathyroidectomised + TE	8	43.14 ± 1.41	s	211.11 ± 18.20	
Thyro-parathyroidectomised + PTE	7	60.49 ± 1.47		219.44 ± 26.95	

TABLE XIV

Adrenal Gland - Effect of Thyro-Parathyroidectomy and Administration of TE and PTE on Calcium Concentration and Ca 45 Distribution

Group of Animals	No. of Animals	Calcium Concentration μg/gm Wet Weight	Sig.	Specific Activity	Sig.
		$\bar{X} \pm$ SEM		$\bar{X} \pm$ SEM	
Normal Control	20	60.73 ± 3.18		1009.83 ± 62.88	
Normal + TE	8	63.14 ± 1.77		932.44 ± 44.27	S
Normal + PTE	8	61.74 ± 1.94	S	747.14 ± 26.89	
Thyro-parathyroidectomised	8	49.84 ± 1.71		833.55 ± 46.67	
Thyro-parathyroidectomized + TE	8	49.71 ± 1.38	S	838.44 ± 25.49	
Thyro-parathyroidectomized + PTE	7	65.08 ± 3.56		764.91 ± 41.27	

decrease in specific activity in the normal animals receiving PTE.

C. Dorsolateral Prostate: (Table XV)

Thyroparathyroidectomy or administration of TE or PTE showed no change in either calcium concentration or specific activity in dorsolateral prostate.

D. Ventral Prostate: (Table XVI)

Thyroparathyroidectomy caused no significant effect in calcium concentration in the ventral prostate. Administration of either PTE or TE to the thyroparathyroidectomised rats or to the normal rats did not produce any change in calcium concentration.

The specific activity in the ventral prostate of thyroparathyroidectomised rats was apparently the same as that in the normal animals. Administration of PTE or TE to the thyroparathyroidectomised rats did not produce any effect. Administration of PTE to the normal animals produced a significant decrease in specific activity. TE showed no effect when administered to the normal animals.

TABLE XVI

Dorsolateral Prostate - Effect of Thyro-Parathyroidectomy and Administration of TE and PTE on Calcium Concentration and Ca^{45} Distribution

Group of Animals	No. of Animals	Calcium Concentration μg/gm Wet Weight	Sig.	Sepcific Activity	Sig.
		$\bar{X} \pm$ SEM		$\bar{X} \pm$ SEM	
Normal Control	20	97.10 ± 6.18		219.90 ± 17.17	
Normal + TE	8	82.75 ± 3.09		255.79 ± 21.45	
Normal + PTE	8	85.17 ± 2.35		172.37 ± 11.19	
Thyro-parathyroidectomised	8	89.43 ± 3.23		169.71 ± 12.25	
Thyro-parathyroidectomised + TE	8	87.91 ± 2.96		156.58 ± 17.14	
Thyro-parathyroidectomised + PTE	7	102.26 ± 3.48		206.36 ± 12.05	

TABLE XVII

Ventral Prostate - Effect of Thyro-Parathyroidectomy and Administration of TE and PTE on Calcium Concentration and Ca-45 Distribution

Group of Animals	No. of Animals	Calcium Concentration $\mu g/gm$ Wet Weight	Sig.	Specific Activity	Sig.
		$\bar{X} \pm$ SEM		$\bar{X} \pm$ SEM	
Normal Control	20	40.63 ± 2.99		384.13 ± 20.98	
Normal + TE	8	34.69 ± 1.56		292.53 ± 21.11	S
Normal + PTE	8	36.31 ± 1.73		227.16 ± 8.39	
Thyro-parathyroidectomised	8	34.89 ± 1.58		369.44 ± 28.33	
Thyro-parathyroidectomised + TE	8	35.16 ± 1.75		261.96 ± 23.29	
Thyro-parathyroidectomised + PTE	7	46.41 ± 1.61		428.16 ± 18.90	

DISCUSSION

A. Evaluation of the methods:

It was early realized that in biological studies where Ca^{45} is used and where the accumulation of the tagged element in the tissues is very low, the sensitivity of the measurement depends to a very large extent upon the ratio of the amount of calcium to the total mass of the sample. In addition, the relatively low β-energy of Ca^{45} required that particular care be taken to obtain a satisfactory measurement of the radioactivity.

In the present investigation it was important to be able to measure calcium concentration chemically and the Ca^{45} concentration by the radioactive assay on the same sample. Since the total amount of tissue available in some of the tissues used in the present work was of the order of 30 to 40 mg (adrenal and prostate) and the total amount of calcium present in these tissues was also of a very low order of magnitude; the combined chemical and radio assay on the same sample posed a particularly difficult problem. The actual amount of calcium to be measured was approximately 2 to 5 μg. The chemical estimation by the classical method of precipitation of calcium through the use of oxalate ions and subsequent estimation by permanganometry was not possible. The precipitation method employed in this study and reported in the methods was found to be superior to the direct measurement of the radioactivity of the ashed tissue. Furthermore, this method had the

advantage of allowing a combined radio-assay and chemical assay of calcium in the same sample; the latter employing spectrophotometry.

The murexide reagent resulted in the formation of a violet complex with calcium which was sufficiently stable under regulated conditions of hydrogen-ion concentration to permit satisfactory estimation. The sample was then quantitatively precipitated by the addition of 95% alcohol to give calcium murexide which could be plated out in a satisfactory fashion on stainless steel planchetts for the determination of the radioactivity. The experiments presented in the methods and published (147) confirmed the soundness of this method. (See page 25)

B. Effect of hormones on calcium metabolism.

Hypophysectomy or the administration of pituitary hormones to the hypophysectomised animals did not have any effect on the calcium concentration of blood. This result concurs with the reported results of Collip (148) and Anderson and Oastler (98) that the plasma level of calcium in hypophysectomised rats is normal. When hypophysectomised rats were given Ca^{45} intravenously the blood level of the radio isotope was almost twice as high as in the normal. This would indicate that there must have been an increase in calcium ion concentration for there to be an increase in the radio isotope level, or, if there is not an increase in the concentration there must be a change in the rate of renewal of the calcium in the tissue. In fact there must be for this specific period of time an increase in the rate of uptake for an increase in the Ca^{45} to occur. Similarly when growth hormone was administered to the hypophysectomised rats, the increased radio isotope concentration in the blood,

caused by hypophysectomy, returned to normal indicating that the change in the rate of uptake of calcium ion has returned to normal under the influence of the administered growth hormone. Ulrich and his co-workers (104) found similar results in calcium concentration and an increased isotope level in the blood and decreased uptake by bone, of hypophysectomised rats. They have also reported (104) that growth hormone administered to hypophysectomised rats receiving Ca^{45} decreased the elevated serum radio isotope level to normal, and increased the skeletal uptake of Ca^{45}. In addition, growth hormone administration reduced to normal the increased fecal excretion of the isotope observed in hypophysectomised animals.

The results obtained from the study of adrenal glands are of particular interest. Hypophysectomy caused a significant increase of calcium concentration which may either be due to weight change in the adrenal gland (adrenal atrophy following hypophysectomy, as measured by weight of the gland) or an actual increase in calcium concentration in the gland. Since ACTH was administered intraperitoneally for a period of four hours as a single dose, it is unlikely that the correction effect as observed by the influence of ACTH administration was due to the trophic action of ACTH on adrenals as measured by increase in adrenal weight. This would, therefore, indicate that hypophysectomy caused an actual increase in calcium concentration in the adrenal which was corrected by ACTH administration.

In the hypophysectomised animals there is a rapid decrease in function of the adrenal cortex and production of adrenal corticosteroids. The above result would therefore indicate that calcium takes part in some way in adrenal corticoid secretions, as there was a build up of calcium

in the adrenals of hypophysectomised rats. Moreover, calcium itself, in certain conditions is known to increase permeability of cell membrane (149) which suggests that it may serve as a stimulus for the release of corticoids. This statement is further substantiated by the fact that normal animals given ACTH showed an increased calcium concentration in the gland, indicating that more adrenal corticosteroid release is necessary to inhibit the excess circulating ACTH. Intravenous administration of Ca^{45} caused a higher radio isotope level in the adrenals of normal animals than the other tissues examined, indicating a possible important role of calcium in the normal functioning of the gland. In hypophysectomised rats there would appear to be an actual increase in calcium concentration in the gland. The addition of Ca^{45} consequently resulted in a lower specific activity. This may also be due to the increased Ca^{45} uptake by the circulating blood. The latter seems to be more logical, because the administration of STH to the hypophysectomised rats caused the blood radio isotope level to be decreased and the adrenal radio isotope level increased to that of normal. The study of ACTH and STH on the Ca^{45} uptake to the normal animals also is in support of this view.

The study of the effect of ACTH and STH on the calcium concentration and Ca^{45} distribution in the dorsolateral and ventral prostate of both normal and hypophysectomised rats suggests that these two hormones play important roles in the metabolism of calcium in those tissues.

Since the administration of ACTH and STH to the hypophysectomised rats resulted in a correction effect of the lesion produced by hypophysectomy in both dorsolateral and ventral prostate, it would appear that

the maintenance of normal calcium concentration in those tissues is controlled by the pituitary gland. The nature of the correction effect however, might be indicative of the maintenance of a proper balance in the calcium concentration between dorsolateral prostate and ventral prostate.

Hypophysectomy resulted in a significant increase in specific activity in the dorsolateral and ventral prostates. This would suggest that the rate of renewal of calcium in these tissues must have increased in the absence of pituitary gland. Administration of ACTH to the hypophysectomised rats does not seem to have any effect on the rate of uptake in these tissues. Growth hormone administration however, seemed to have a partial effect at least on the rate of renewal of calcium but in the dorsolateral prostate only. The effect was in the direction of the levels found in the hypophysectomized control animals.

In liver and heart tissue, neither hypophysectomy nor the administration of ACTH or STH to the hypophysectomised rats or to the normal rats had any effect on the calcium concentration or specific activity. This would seem to be indicative of the independence of the normal metabolism of calcium in these tissues in so far as the pituitary gland is concerned.

The study of the effect of adrenalectomy and administration of DCA to the adrenalectomised rats on the calcium concentration and Ca^{45} distribution suggests that in the absence of the adrenal gland, calcium is mobilized to a greater extent from the tissues of the body and the level in the blood increased over that which was obtained when the adrenals are present. Administration of DCA appears to prevent completely the

effect of adrenalectomy. The radio isotope level in the blood is also increased as a result of adrenalectomy and this also appears to return toward the normal radio isotope level under the influence of DCA.

It is generally accepted (150) that ACTH from pituitary causes maintenance of the adrenal gland. The adrenal then produces the corticosteriods and the level of the steriods in the blood serves as a control on the pituitary to cause a change in the output of ACTH. This "feedback" system is thus adjusted so that there is normally a controlled output of ACTH which in turn causes the adrenal to produce the normal requirement of steroids. Consequently if the adrenal is removed, the output of ACTH increases and there is an excess of ACTH in the blood. A similar situation will occur when exogenous ACTH is administered to normal animals.

In the discussion of the hypophysectomised series it has been suggested that calcium is somehow related to the release of steroid hormones from the adrenal gland. On that basis, one would therefore expect that in the absence of the adrenal gland, there will be a temporary build up of calcium in the blood as was found. Also the increased rate of uptake of Ca^{45} in the blood of the adrenalectomised rat appears to fit this concept. Administration of DCA to the adrenalectomised rat caused the expected effect on the production of ACTH from the pituitary resulting in the correction effect.

Adrenalectomy caused a decrease in calcium concentration in the dorsolateral prostate. This would suggest that in the absence of the adrenal gland clacium is withdrawn or lost from this organ to a greater extent than when the adrenals are present. Administration of DCA to

the adrenalectomised rats did not appear to have any effect on the lesion produced by adrenalectomy. The increased specific activity observed in this tissue in adrenalectomised rats probably resulted from the decrease in calcium concentration and was similarly not corrected when DCA was administered.

The metabolism of calcium in the ventral prostate is also affected by adrenalectomy. Although there seemed to be no change in the concentration of calcium in the tissue after adrenalectomy, there appeared to be a significant decrease in specific activity in the tissue of adrenalectomised rats. This would indicate that in the absence of the adrenal gland the rate of renewal of calcium in the tissue has decreased significantly. Administration of DCA to either the normal or adrenalectomised rats did not seem to have any effect either in calcium concentration or Ca^{45} uptake.

In the absence of the adrenal gland, calcium metabolism in liver and heart seemed to be normal. The administration of DCA to the adrenalectomised rat did not seem to have any effect on calcium concentration and Ca^{45} distribution in these tissues. However, when normal animals were administered DCA, there was a significant increase in the calcium concentration.

Although the normal calcium balance in the body will be affected by various factors such as diet, absorption and excretion, and the ebb and flow of the mineral between bone and blood, there is normally an excellent homeostatic control maintaining the plasma calcium concentration within a very narrow limit. The principle factors concerned are the skeleton with its vast reserve of calcium and the parathyroid gland

which together serve as "calciostats" (118). Ultimately, normal calcium balance must depend on net movement of calcium into or out of the stable bone mineral reserve.

Since changes in calcium concentration in certain soft tissues of both hypophysectomised and adrenalectomised animals were observed, it might indicate that the metabolism of calcium in those tissues were affected by the absence of the pituitary and/or the adrenal gland. Moreover, since all the animals in the above two series of experiments had intact and functioning parathyroid glands, it has been necessary to study the effect of thyroparathyroidectomy and administration of PTE and TE to further evaluate and clarify the above observations.

Changes produced by parathyroidectomy and by the administration of PTE have been extensively investigated by many workers. As early as 1925, Collip (115) had confirmed that hypocalcemia resulted from parathyroidectomy and that this condition could be corrected by the administration of PTE. MacLean (118) has proved beyond doubt that the calcium concentration in the blood is under the control of the parathyroid gland. Thus, in our observation, the significant decrease in the calcium concentration of the blood is an expected one and was considered as the criteria of the success of parathyroidectomy. The increased rate of uptake of radio calcium by the blood of the thyroparathyroidectomised rats probably resulted by an increase in the movement of calcium from the "labile" calcium storage pool (easily exchangable when blood level is lowered) in bone towards the blood. Administration of PTE restores the normal calcium level in blood and also permitted the mobilization of calcium to return to normal. Administration of TE to the thyroparathyroidectomised or normal animals has no effect on either the calcium

concentration or the Ca^{45} distribution in blood.

Thyroparathyroidectomy resulted in a significant decrease in calcium concentration in the adrenal gland. The normal concentration was restored by the administration of PTE as was seen in the blood. However, the rate of renewal of calcium in the gland did not seem to be affected by thyroparathyroidectomy as no change resulted in the specific activity. The increased rate of uptake of Ca^{45} in the tissue of normal animals receiving PTE suggest that the hormone mobilizes Ca^{45} and tends to raise the normal concentration of calcium in the gland. Administration of TE to the thyroparathyroidectomised or normal animals resulted in no effect on either the calcium concentration or the Ca^{45} distribution in adrenal gland.

The calcium concentration and Ca^{45} activity in the dorsolateral and ventral prostate of thyroparathyroidectomised animals showed no significant difference from that in normal animals. This would suggest that the metabolism of calcium in these tissues is probably independent of the thyroid and parathyroid. However, in ventral prostate, administration of PTE to the normal animal caused a significant decrease in specific activity suggesting that this hormone mobilizes calcium and tends to lower the normal calcium balance in the tissue.

SUMMARY AND CONCLUSIONS

1. A simple and convenient method for the combined chemical analysis and radio assay of microgram amounts of calcium and administered calcium^{45} in soft tissues has been presented. This method was found to be accurate and reproducible.
2. A study was made of the effects of hypophysectomy, adrenalectomy and thyroparathyroidectomy on calcium concentration and Ca^{45} distribution in soft tissues of male rats.
3. Treatment of normal and experimental animals with ACTH, STH, DCA, PTE and TE was also undertaken.
4. Hypophysectomy or administration of pituitary hormones to the hypophysectomised rats did not have any effect on the calcium concentration of blood. When hypophysectomised rats were given Ca^{45} intravenously, the blood level of the radioisotope was almost twice as high as in the normal animals. This effect was reversed by the influence of administered growth hormone.
5. Hypophysectomy caused an actual increase in calcium concentration in the adrenal gland which was corrected by the influence of ACTH administration. Administration of STH markedly increased the lowered Ca^{45} activity observed in the hypophysectomised rat to about the level apparent in normal animals. It has been suggested that calcium takes part in someway in adrenal corticoid secretions.
6. In dorsolateral prostate and ventral prostate the biochemical lesion produced by hypophysectomy was alleviated by the administration of ACTH and STH. The nature of the correction effect might be

indicative of the maintenance of a proper balance in the calcium concentration between dorsolateral and ventral prostate.

7. The study of the effect of hypophysectomy and administration of ACTH and STH on the calcium concentration and Ca^{45} incorporation in liver and heart suggests that the normal metabolism of calcium in these tissues is independent of the pituitary gland.

8. Adrenalectomy caused an increase in calcium concentration and Ca^{45} activity in blood. Administration of DCA appeared to prevent completely the effect of adrenalectomy.

9. Adrenalectomy caused a decrease in calcium concentration and increase in Ca^{45} activity in the dorsolateral prostate. Administration of DCA to the adrenalectomised rats did not produce any significant effect.

10. The metabolism of calcium in the ventral prostate is also affected by adrenalectomy. In the absence of the adrenal glands the rate of renewal of calcium in the tissue decreased significantly. Administration of DCA resulted in no effect.

11. Thyroparathyroidectomy caused a significant decrease in calcium concentration in blood and adrenal gland which was corrected by the administration of parathyroid extract.

12. The calcium concentration and Ca^{45} activity in the dorsolateral and ventral prostate of the thyroparathyroidectomised animals showed no significant difference from that in normal animals. This would suggest that the metabolism of calcium in these tissues is independent of the thyroid and parathyroid.

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A P P E N D I C E S

I. Normal Animals

	Whole Blood		Adrenal		Dorsolateral Prostate		Ventral Prostate		Liver		Heart	
	Ca*	Sp.A.	Sp.A.	Ca*	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.
	44.73	106.28	1252.10	52.85	135.48	190.45	67.53	472.90	45.52	336.21	44.36	281.35
	54.00	102.40	1478.23	66.53	151.80	308.71	44.66	450.30	57.69	257.57	75.81	187.08
	82.18	114.58	1023.40	54.83	78.68	174.10	83.13	517.71	42.58	275.17	38.70	322.58
	64.83	114.65	716.88	58.56	41.60	249.75	30.36	428.38	30.05	325.28	30.69	244.76
	64.83	259.67	1095.70	35.56	130.60	126.90	38.04	281.42	44.60	258.38	33.69	306.45
	74.51	317.22	567.74	75.50	94.65	271.14	35.49	371.14	23.94	548.78	40.88	358.57
	71.42	146.37	1050.00	50.78	124.55	304.57	38.71	538.80	48.76	218.17	36.75	258.81
	56.00	163.80	1431.76	90.90	71.79	210.00	31.71	538.80	48.76	218.17	36.75	454.24
	81.57	198.93	808.00	52.90	103.30	460.64	30.69	528.88	24.03	333.20	32.31	277.33
	79.08	115.09	1616.00	48.57	104.63	172.07	38.00	505.48	44.60	284.83	33.04	359.55
	55.10	262.26	572.75	71.42	127.60	194.93	32.29	398.85	20.85	507.05	64.63	186.91
	30.36	337.23	1042.53	96.34	58.40	135.52	38.41	420.48	47.62	315.48	36.86	353.54
	42.71	108.26	862.48	66.42	98.43	218.42	45.62	335.40	44.13	205.82	97.35	187.92
	60.25	120.10	742.39	58.41	90.41	196.34	49.80	289.56	24.37	606.34	69.86	380.85
	61.83	136.42	1061.44	60.01	81.50	234.43	36.25	264.51				
	58.96	209.43	918.43	54.21	68.40	134.80	30.12	401.20				
	64.42	186.21	1010.36	59.40	102.83	262.51	31.51	396.12				
	62.18	174.50	1151.66	49.61	103.92	187.50	29.65	294.44				
	57.63	141.88	938.40	53.50	78.62	169.33	38.10	218.53				
	51.64	180.46	856.39	58.40	94.83	194.87	42.62	264.40				
Mean	60.92	174.89	1009.83	60.73	97.10	219.90	40.63	384.13	37.60	346.81	46.65	297.14
S.D.	13.15	66.79	280.95	14.23	27.73	76.79	13.39	93.79	11.88	123.40	20.02	80.53
SEM(±)	2.94	14.94	62.85	3.18	6.18	17.17	2.99	20.98	3.17	32.99	5.35	21.53
μ (±)	6.31	32.06	134.86	6.83	13.26	36.86	6.42	45.02	7.13	74.04	12.01	48.32

* calcium concentration μg/gm wet weight.

II. Normal Animals receiving ACTH

	Whole Blood		Adrenal		Dorsolateral Prostate		Ventral Prostate		Liver		Heart	
	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.
	64.74	265.77	65.61	493.60	62.64	528.88	68.10	290.37	36.83	310.37	48.66	266.45
	70.69	304.33	74.58	564.44	72.76	563.87	58.71	363.22	38.65	304.83	48.38	244.84
	67.64	271.55	98.41	511.93	57.83	350.64	46.40	380.68	45.12	255.48	49.23	227.42
	51.56	221.30	54.11	462.80	55.21	481.11	66.08	240.00	36.60	323.60	32.53	264.83
	57.68	217.40	70.12	467.77	62.62	508.71	44.61	267.86	36.38	268.71	45.96	240.57
	66.28	258.26	77.30	417.74	80.31	548.71	44.61	415.52	36.38	268.71	45.96	240.57
	43.95	199.81	72.68	571.03	62.67	534.22	52.06	196.89	33.88	296.66	25.09	216.30
	49.28	189.82	68.35	442.96	49.02	458.80	49.17	267.86	27.41	325.92	60.32	367.21
	55.48	314.68	76.45	518.00	62.06	539.62	60.03	297.71	45.25	309.67	47.14	247.50
	59.38	299.74	70.22	634.80	61.50	625.18	42.99	314.19	45.66	343.44	47.07	243.63
	60.12	298.71	76.48	475.92	58.00	615.00	47.30	363.79	45.52	260.92	51.92	278.06
	58.24	258.65	73.12	505.20	62.24	506.00	53.35	304.20				
	60.62	279.30	74.28	516.48	61.50	508.30	54.60	313.95				
	69.71	233.64	75.64	493.90	69.40	556.28	59.42	285.60				
Mean	59.67	258.07	73.38	505.47	62.70	523.24	53.88	307.27	38.70	298.90	44.62	263.00
S.D.	7.84	40.26	9.38	56.03	7.59	67.41	7.73	58.30	6.01	28.22	9.97	41.35
SEM(±)	2.10	10.76	2.51	14.98	2.03	18.02	2.07	15.59	1.81	8.51	3.01	12.46
μ (±)	4.70	24.16	5.63	33.62	4.55	40.45	4.70	34.98	4.23	19.88	7.02	29.14

* calcium concentration μg/gm wet weight.

III. Normal Animals Receiving Growth Hormone

	Whole Blood		Adrenal		Dorsolateral Prostate		Ventral Prostate		Liver		Heart	
	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.
	58.23	175.30	120.62	1021.43	126.61	368.43	42.66	498.21	38.92	384.14	56.42	380.12
	49.51	169.42	101.43	1084.56	98.44	294.36	39.86	504.62	40.62	284.35	38.45	640.18
	63.18	194.51	96.60	976.45	105.32	318.47	50.42	532.43	38.44	364.51	29.38	480.23
	65.20	201.42	120.01	994.31	113.66	364.13	57.61	380.40	48.43	394.12	50.85	364.12
	62.19	292.40	112.62	1141.42	119.50	318.25	52.81	536.31	50.67	380.19	38.61	454.15
	59.42	164.52	80.51	1261.59	150.82	218.94	62.27	538.92	48.30	450.58	48.42	384.44
	65.20	174.23	88.33	1264.50	148.61	301.44	50.43	468.47	39.10	467.55	39.33	363.39
	58.13	194.28	98.62	984.22	112.83	322.62	38.92	380.43	36.25	398.12	41.12	494.60
	60.46	188.33	101.43	862.40	121.62	384.44	39.41	398.40	40.42	284.25	47.78	267.48
	67.43	175.96	84.32	843.80	96.40	284.91	30.42	528.44	50.88	363.44	48.95	376.33
	69.28	162.42	84.36	968.41	97.95	288.80	50.62	504.39	54.63	276.31	60.04	484.81
	50.51	174.40	94.52	1021.32	101.43	264.51	58.12	487.46	39.12	331.95	57.83	364.51
	61.36	180.36	101.62	1043.65	94.36	369.30	47.16	479.72				
	67.89	134.66	93.61	872.70	99.85	294.30	45.20	406.25				
	52.52	205.21	97.50	962.50	80.62	298.60	49.34	580.26				
	49.96	189.62	102.10	1082.88	104.21	305.80	58.62	394.60				
	68.56	177.40	88.63	977.70	107.34	318.60	50.18	415.60				
	60.21	196.66	87.50	1086.23	115.44	298.31	39.62	480.60				
	63.63	174.20	94.30	794.33	99.60	212.61	43.81	492.30				
	69.62	189.30	92.40	786.29	94.38	294.50	40.62	484.60				
Mean	61.12	185.73	97.05	1001.53	109.45	306.07	47.40	469.62	43.82	364.99	46.43	421.20
S.D.(±)	6.47	19.01	10.96	132.55	17.57	44.41	8.15	53.73	6.26	62.11	9.24	95.56
SEM(±)	1.45	4.25	2.45	29.65	3.93	9.94	1.82	12.02	1.81	17.95	2.67	27.62
μ (±)	3.10	9.12	5.26	63.62	8.43	21.32	3.91	25.79	4.16	41.24	6.14	63.45

* calcium concentration μg/gm wet weight

IV. Normal Animals Receiving DCA

	Whole Blood		Adrenal		Dorsolateral Prostate		Ventral Prostate		Liver		Heart	
	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.
	60.54	418.71	51.76	845.36	51.76	766.82	38.92	603.19	35.77	383.11	34.28	451.71
	68.22	356.00	79.27	683.55	79.27	493.22	45.63	590.66	54.86	318.38	44.99	256.16
	59.42	346.57	56.16	781.91	56.16	583.41	58.82	403.44	50.57	374.19	43.36	287.41
	57.51	403.22	59.05	499.60	59.05	636.22	45.73	491.55	45.36	308.92	35.65	412.44
	54.91	356.36	84.39	593.68	84.39	460.30	48.82	487.60	43.47	263.22	44.09	292.42
	48.40	326.96	47.56	653.33	47.56	512.19	84.61	313.93	48.52	301.82	32.30	271.93
	70.99	374.85	47.59	799.51	47.59	678.19	40.92	512.19	35.32	362.66	44.10	442.48
	70.62	318.93	80.52	508.80	70.25	365.81	45.13	464.68	47.62	321.72	43.54	316.12
	57.28	331.42	45.52	533.44	52.68	636.17	60.19	400.00	47.00	289.65	31.01	336.35
	65.29	414.00	79.69	920.00	45.52	673.19	47.68	507.80	52.63	304.19	31.20	405.36
	56.39	462.68	63.61	924.53	43.61	516.77	59.94	409.31	47.30	332.45	52.96	427.55
	56.79	376.66	61.56	771.74	70.25	442.40	58.11	443.10	50.66	381.29	45.96	347.58
	60.53	314.14	68.42	852.38	63.61	560.21	52.87	468.95				
	61.84	380.92	69.62	439.40	61.56	394.29	40.34	670.28				
	63.24	328.64	58.20	468.30	62.52	494.28	54.62	570.28				
	58.95	374.50	51.54	494.39	60.92	608.26	54.01	468.22				
	67.34	405.80	60.28	568.40	58.52	564.28	59.62	394.21				
	64.38	415.92	59.29	430.94	50.18	550.27	51.40	360.80				
	60.20	350.16	60.80	482.60	60.20	549.94	49.60	404.14				
	59.42	318.90	59.30	692.02	62.18	560.29	50.24	418.29				
Mean	61.11	368.77	62.21	647.19	59.39	552.58	52.36	469.13	46.59	328.49	40.29	353.96
S.D.	5.57	41.22	11.55	166.74	9.55	98.99	10.10	88.62	6.01	38.90	7.09	70.81
SEM	1.25	9.22	2.58	37.30	2.14	22.14	2.26	19.82	1.74	11.24	2.05	20.46
u (±)	2.67	19.78	5.54	80.04	4.58	47.52	4.85	42.54	3.99	25.83	4.71	47.02

* calcium concentration ug/gm wet weight

V. Normal Animals Receiving PTE

	Whole Blood		Adrenal		Dorsolateral Prostate		Ventral Prostate	
	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.
	68.32	405.3	62.18	751.5	83.61	187.4	39.5	236.5
	65.94	350.6	70.49	682.6	79.52	138.5	38.6	281.5
	70.84	398.0	51.25	801.6	84.63	196.2	29.5	255.2
	63.40	561.5	61.81	859.7	92.18	184.5	40.2	165.1
	72.51	489.6	59.64	625.1	88.58	112.5	41.5	212.5
	59.18	364.8	62.00	733.7	72.51	164.5	33.6	187.2
	60.25	418.4	66.12	812.5	91.81	194.2	38.5	194.2
	62.50	409.0	60.40	710.4	88.52	201.2	29.1	285.1
Mean	65.36	424.58	61.74	747.14	85.17	172.37	36.31	227.16
SD (±)	4.89	69.48	5.48	76.04	6.65	31.62	4.89	23.74
SEM (±)	1.73	24.57	1.94	26.89	2.35	11.19	1.73	8.39
μ (±)	4.375	62.128	4.895	67.994	5.936	28.262	4.375	21.214

* calcium concentration μg/gm wet weight

VI. Normal Animals Receiving TE

	Whole Blood		Adrenal		Dorsolateral Prostate		Ventral Prostate	
	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.
	61.28	133.8	68.5	852.4	88.35	218.4	39.40	281.5
	60.15	162.5	60.1	962.1	90.81	264.2	30.42	392.4
	58.23	180.2	59.1	1024.1	68.21	308.9	33.51	202.5
	62.94	194.2	64.3	991.1	94.29	196.4	38.43	310.2
	64.81	155.6	66.4	852.5	74.82	318.2	40.81	312.6
	52.51	133.8	69.4	1162.5	86.16	198.4	29.61	228.2
	66.21	120.9	62.5	825.1	80.41	262.4	31.51	238.6
	55.20	169.1	54.8	789.7	79.19	279.4	33.81	294.2
Mean	60.16	156.26	63.14	932.44	82.75	255.79	34.69	292.53
SD (±)	4.69	25.28	5.00	125.2	8.74	60.67	4.42	59.70
SEM	1.66	8.94	1.77	44.27	3.09	21.45	1.56	21.11
μ	4.19	22.60	4.46	111.94	7.81	54.25	3.952	53.38

* calcium concentration μg/gm wet weight

VII. Hypophysectomised Control

	Whole Blood		Adrenal		Dorsolateral Prostate		Ventral Prostate		Liver		Heart	
	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.
	63.40	401.77	92.43	800.92	38.21	739.33	59.93	535.82	36.87	611.64	48.95	236.00
	40.27	584.37	124.52	606.06	59.50	654.78	67.89	326.87	38.56	347.27	84.51	208.81
	52.24	367.14	87.44	1022.87	39.57	703.06	55.93	295.45	50.73	376.55	41.32	503.72
	64.28	574.16	107.65	823.11	67.89	975.61	45.19	672.87	48.69	294.57	59.04	271.93
	76.11	591.39	105.14	628.41	55.93	416.40	70.64	483.93	50.41	391.29	75.23	255.18
	70.12	580.51	118.42	760.89	85.19	529.75	72.88	318.78	36.23	395.16	61.27	692.00
	59.02	484.00	94.90	870.89	87.41	1012.90	48.81	886.70	48.17	433.78	63.01	263.22
	48.54	495.51	120.44	628.42	72.95	548.69	64.42	444.19	45.65	373.94	64.45	214.68
	52.54	447.74	79.48	1094.40	57.84	530.22	72.29	252.66	44.86	397.93	52.23	586.24
	40.99	539.14	105.38	944.22	67.08	934.63	44.13	650.99	42.92	522.34	45.43	347.58
	73.60	347.17	115.18	844.93	56.08	628.38	73.23	453.79	47.20	384.83	46.66	252.28
	64.27	405.56	124.30	671.22	51.12	498.47	67.48	484.20	39.65	343.87	61.61	602.40
	60.15	494.62	115.60	689.60	98.10	717.48	54.28	512.60				
	67.25	389.34	102.94	770.28	70.22	615.20	59.04	594.20				
	59.18	294.32	112.62	725.62	63.79	912.15	64.12	604.18				
	51.26	564.20	98.24	815.23	58.24	718.17	69.40	518.20				
	66.12	402.12	93.69	515.85	67.30	610.12	70.20	615.74				
	67.82	418.16	84.28	590.30	68.90	985.77	56.65	690.14				
	59.15	439.40	124.62	605.95	55.16	725.33	50.13	484.20				
	59.60	462.17	102.95	618.20	52.25	833.17	47.74	520.18				
Mean	59.80	464.14	105.51	751.37	63.64	714.48	60.72	517.28	44.16	406.12	58.64	369.50
S.D.	9.29	87.63	13.88	153.26	14.86	177.10	9.86	152.09	5.26	84.77	12.67	175.54
SEM	2.19	19.60	3.10	34.29	3.32	39.62	2.21	34.02	1.52	24.47	3.66	50.66
u (±)	4.70	42.06	6.66	73.56	7.13	85.01	4.73	73.00	3.49	56.29	8.41	116.56

* calcium concentration $\mu g/gm$ wet weight

VIII. Hypophysectomised Rats Receiving ACTH

	Whole Blood		Adrenal		Dorsolateral Prostate		Ventral Prostate		Liver		Heart	
	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.
	73.41	146.21	96.07	404.08	85.87	475.79	28.29	896.63	44.86	322.58	49.02	574.00
	56.79	233.67	74.28	627.40	80.48	362.42	40.62	599.03	37.93	425.60	47.75	267.27
	99.77	260.60	76.27	1041.00	76.29	337.05	45.96	422.34	33.20	454.40	51.58	461.66
	101.28	177.19	35.46	915.75	147.60	783.50	38.82	477.40	42.93	316.00	77.05	655.55
	86.81	189.45	64.28	854.66	105.08	840.00	37.29	731.90	70.85	308.28	51.75	277.75
	46.69	227.34	72.58	528.88	116.98	268.71	25.10	652.80	82.85	379.65	82.11	608.44
	65.95	194.13	80.64	554.22	77.32	651.55	20.83	388.00	52.54	316.63	35.05	292.00
	48.24	308.01	96.52	666.80	122.80	297.85	85.35	659.55	61.14	221.44	61.14	277.41
	61.81	340.00	91.42	422.23	90.29	558.90	34.08	376.44	30.71	534.04	45.43	489.21
	80.28	280.05	80.34	470.40	104.72	628.81	41.42	837.20	49.91	892.00	27.24	409.04
	49.38	375.81	74.90	839.40	91.52	752.15	41.44	414.51	73.13	222.21	67.34	270.00
	50.64	248.27	72.53	561.67	80.62	650.96	54.08	761.82	52.73	204.86	54.04	316.15
	57.42	250.31	68.91	685.32	100.57	684.18	29.14	421.81				
	68.21	264.15	61.52	739.51	75.39	739.24	38.39	396.50				
	60.21	218.52	70.28	797.85	110.70	518.50	48.62	318.17				
	65.15	196.27	80.58	739.65	90.64	690.56	40.04	515.18				
Mean	67.00	244.37	74.79	678.05	97.30	577.51	40.60	554.46	52.75	399.81	54.12	403.24
S.D.	17.27	60.84	14.67	183.46	20.01	182.11	14.72	181.88	16.40	159.11	15.87	109.32
SEM	4.32	15.21	3.67	45.86	5.00	45.53	3.68	45.47	4.73	45.93	4.58	31.56
u (±)	9.50	33.46	8.07	100.90	11.00	100.16	8.10	100.03	10.89	105.65	10.54	72.59

* calcium concentration µg/gm wet weight

IX. Hypophysectomised Rats Receiving Growth Hormone

	Whole Blood		Adrenal		Dorsolateral Prostate		Ventral Prostate		Liver		Heart	
	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.
	58.61	150.23	110.01	864.21	98.25	315.18	46.15	398.62	40.62	460.20	51.25	368.99
	60.21	189.51	115.21	952.18	70.18	320.45	50.58	450.18	51.21	580.12	52.51	298.25
	54.13	252.01	110.21	760.23	102.51	298.41	40.38	418.30	48.63	386.21	48.14	305.66
	65.60	196.21	98.61	1005.66	116.52	270.52	43.50	296.15	38.41	294.41	39.21	333.17
	80.92	174.04	80.24	895.23	93.80	390.81	45.91	284.51	42.51	315.21	41.61	298.45
	60.80	183.63	101.61	1251.50	80.38	400.62	51.53	518.06	39.61	301.44	52.14	250.21
	52.67	194.36	105.21	723.62	85.15	258.18	41.39	448.40	34.22	240.38	39.26	302.44
	54.84	158.41	114.62	895.88	110.25	320.31	39.41	381.62	42.63	540.08	34.13	265.87
	62.16	138.81	160.23	998.91	113.89	348.15	45.36	341.51	45.18	260.21	50.21	394.29
	56.34	140.60	84.72	920.43	75.62	218.50	51.80	387.81	35.16	360.42	55.61	240.44
	58.90	281.01	94.52	726.11	96.15	396.53	54.39	317.41	39.90	402.18	60.02	364.27
	59.20	164.61	102.51	858.20	101.23	325.45	60.40	305.21	40.20	380.25	40.21	395.52
	60.42	185.35	106.56	904.62	83.61	318.41	38.42	516.32	41.51	396.66	48.04	248.98
	58.42	145.64	98.31	1003.18								
	51.69	196.38	96.62	925.51								
	67.55	158.64	104.35	856.28								
	60.60	201.38	103.89	1162.85								
	61.22	205.94	108.60	958.14								
	70.40	184.36	84.39	928.62								
	53.15	250.15	103.64	1061.25								
Mean	60.38	187.56	104.20	932.63	94.43	321.66	46.86	389.55	41.52	378.21	47.10	312.81
S.D.	6.82	38.17	16.28	130.34	14.68	54.18	6.54	79.10	4.76	102.03	7.59	54.55
SEM	1.52	8.54	3.64	29.16	4.07	15.02	1.81	21.94	1.32	28.29	2.10	15.13
u (±)	3.27	18.32	7.81	62.56	9.23	34.08	4.11	49.75	2.99	64.18	4.77	34.31

* calcium concentration µg/gm wet weight

X. Adrenalectomised Animals

	Whole Blood		Dorsolateral Prostate		Ventral Prostate		Liver		Heart	
	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.
	80.61	569.52	50.00	385.81	41.01	238.07	34.79	286.77	39.91	313.96
	81.47	410.00	50.07	329.37	44.16	229.92	41.45	556.67	38.99	268.71
	93.87	552.77	53.35	394.19	46.93	160.86	32.19	322.65	33.58	320.47
	73.71	645.10	37.72	309.63	40.46	232.38	30.72	281.61	33.54	315.09
	126.16	498.70	36.09	305.06	25.62	277.74	27.62	371.00	29.37	268.06
	85.34	377.64	50.00	350.32	24.04	382.64	33.65	319.35	52.08	336.01
	69.61	466.98	47.91	343.87	41.10	244.81	38.99	276.77	39.45	311.15
	67.69	586.42	56.94	242.57	44.84	248.83	32.47	314.33	37.37	318.11
	71.67	425.70	45.58	386.77	41.45	235.71	34.79	272.90	38.51	266.45
	88.41	637.34	56.02	376.96	47.37	294.00	38.13	308.38	38.17	260.96
	66.61	507.33	65.86	330.91	39.53	200.48	47.34	276.36	42.82	272.25
	55.42	512.23	53.19	352.42	36.84	206.00	47.75	252.12	29.77	353.24
	72.79	401.36	50.45	401.64	39.04	196.25	36.12	318.51	36.94	416.04
	83.05	482.94	52.92	305.15	40.08	218.97	34.84	390.25	39.81	209.15
Mean	79.82	505.36	50.44	343.90	39.46	240.48	36.49	324.83	37.88	302.12
S.D.	16.49	85.03	11.54	44.28	6.88	52.84	5.83	26.74	5.65	50.00
SEM	4.41	22.74	3.08	16.16	1.84	14.13	1.56	20.52	1.51	13.37
μ (±)	9.89	51.02	6.92	26.57	4.13	31.70	3.50	46.04	3.39	30.00

*calcium concentration μg/gm wet weight

XI. Adrenalectomised Rats Receiving DCA

	Whole Blood		Dorsolateral Prostate		Ventral Prostate		Liver		Heart	
	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.
	82.29	236.45	53.25	271.35	47.31	251.64	45.64	250.60	32.59	324.90
	69.58	288.00	56.38	300.01	40.36	263.57	44.71	266.36	42.23	276.12
	62.10	259.25	67.34	370.30	41.40	352.07	33.26	337.35	38.09	278.06
	64.93	233.43	61.14	350.60	42.33	240.50	32.66	256.67	37.35	252.26
	44.64	177.85	46.54	361.71	45.17	269.25	36.35	368.39	38.82	395.94
	55.18	250.00	37.96	407.16	35.67	293.63	39.25	315.24	44.46	341.92
	73.42	418.98	50.07	289.71	39.07	270.00	45.26	254.54	48.43	287.74
	52.89	385.66	43.42	308.28	64.45	291.81	38.27	279.35	34.01	348.69
	54.09	304.57	55.93	312.42	38.65	343.87	32.59	368.30	44.73	299.67
	56.36	286.36	46.57	417.35	42.22	284.00	34.75	270.32	41.22	268.06
	60.99	252.57	54.82	319.09	50.99	254.42	29.71	325.28	38.04	253.87
	59.32	242.57	46.94	376.95	39.29	304.09	42.09	247.57	41.16	294.83
	61.24	294.62	50.03	289.15	43.98	313.96	37.76	230.92	40.05	218.42
	62.10	304.18	51.50	313.76	40.64	204.84	36.15	315.16	39.62	316.10
Mean	61.37	281.03	51.56	334.84	43.68	287.26	37.75	291.86	40.06	296.97
S.D.	9.35	61.98	7.46	45.83	7.12	39.69	5.11	45.76	4.18	45.68
SEM (±)	2.50	16.57	1.99	12.25	1.90	10.61	1.37	12.24	1.12	12.21
μ (±)	5.61	37.19	4.48	27.50	4.27	23.81	3.07	27.46	2.51	27.41

* calcium concentration μg/gm wet weight

XII. Thyro-Parathyrodectomised Control

	Whole Blood		Adrenal		Dorsolateral Prostate		Ventral Prostate	
	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.
	42.5	258.2	52.8	825.1	88.7	172.5	30.2	418.2
	49.3	334.0	40.5	962.4	97.0	139.1	33.1	442.1
	48.6	264.1	51.4	725.2	90.6	178.2	38.6	300.9
	47.2	402.2	48.6	618.2	86.8	183.6	39.8	234.3
	39.6	318.4	53.4	982.4	98.4	182.1	40.4	481.2
	40.8	289.2	49.6	842.5	72.4	103.2	29.4	384.5
	41.6	178.4	54.2	962.4	82.3	220.4	31.2	364.9
	40.04	264.2	56.2	750.2	99.2	178.6	36.4	329.4
Mean	43.75	288.58	49.84	833.55	89.43	169.71	34.89	369.44
S.D. (±)	3.94	65.59	4.85	131.42	9.13	34.64	4.47	80.12
SEM	1.39	23.19	1.71	46.47	3.23	12.25	1.58	28.33
μ	3.52	58.63	3.35	117.48	8.16	30.96	3.99	71.62

* calcium concentration μg/gm wet weight

XIII. Thyroparathyroidectomised Receiving PTE

	Whole Blood		Adrenal		Dorsolateral Prostate		Ventral Prostate	
	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.
	60.10	326.1	68.23	628.1	96.8	176.2	50.2	428.6
	59.82	289.4	62.41	752.4	113.4	182.5	49.2	421.4
	67.75	264.2	59.20	832.4	110.2	209.5	39.8	371.2
	61.8	183.4	81.40	752.4	101.2	201.2	50.4	518.3
	56.4	196.4	64.26	684.2	95.6	220.4	43.6	442.6
	59.4	201.4	53.43	762.0	108.3	264.3	46.3	394.2
	58.2	185.2	66.61	94.29	90.6	190.4	48.9	420.8
Mean	60.49	219.44	65.08	764.91	102.26	206.36	46.91	428.16
S.D. (±)	3.61	65.95	8.72	100.99	8.52	29.51	3.95	46.26
SEM	1.47	26.95	3.56	41.27	3.48	12.05	1.61	18.90
μ	3.58	65.42	8.65	100.18	8.45	29.27	3.91	45.88

* Calcium concentration µg/gm wet weight

XIV. Thyroparathyroidectomised Receiving TE

	Whole Blood		Adrenal		Dorsolateral Prostate		Ventral Prostate	
	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.	Ca*	Sp.A.
	42.5	218.4	52.4	828.4	90.4	103.2	30.6	288.4
	49.3	154.3	46.9	960.2	80.3	220.4	34.8	325.2
	38.8	263.9	47.3	750.2	78.9	182.6	39.6	340.8
	41.2	194.5	54.9	918.4	90.6	95.5	30.2	305.7
	39.6	138.4	50.4	813.4	86.8	205.5	33.1	184.2
	40.8	208.6	52.1	764.3	98.4	186.3	43.6	197.1
	44.3	216.3	43.6	812.4	78.3	118.4	38.6	220.2
	48.6	294.5	50.1	860.2	99.6	139.2	30.8	264.1
Mean	43.14	211.11	49.71	838.44	87.91	156.33	35.16	261.96
S.D. (±)	3.96	51.48	3.62	72.11	8.39	48.48	4.95	65.88
SEM	1.41	18.20	1.28	25.49	2.96	17.14	1.75	23.29
μ	3.54	46.02	3.22	64.46	7.50	43.24	4.41	58.89

* calcium concentration μg/gm wet weight